Genetics and Agronomy of Transient Salinity In *Triticum durum* and *T. aestivum*

by

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ABSTRACT

Transient salinity in soils is characterised by high concentrations of salts in the subsoil, which vary with depth and change throughout the season in response to rainfall, surface evaporation and water usage by vegetation. Soils affected by this form of salinity comprise at least 50% of the area cropped to cereals in South Australia and have a large impact on grain production, particularly in years receiving below average spring rainfall. Durum wheat (*Triticum turgidum* L. Var *durum*) is less tolerant of transient salinity than locally developed bread wheat (*Triticum aestivum*) varieties, and this results in reliable durum production being restricted to relatively unaffected soils.

Field trials were conducted to assess the relative impact of transient salinity, boron toxicity and bicarbonate on crop production. Despite more than half of the sites being located on subsoils with salinity levels in excess of 4dS/m ECe, there was only one transient saline site, at Roseworthy, where EC was the dominant soil co-variate affecting yield. Boron toxicity was the dominant covariate at another Roseworthy site, while high pH was the dominant factor at half of the locations investigated. At many of the sites, more than one constraint was present at levels expected to restrict crop growth, yet one dominant factor explained most of the variation in yield. This highlighted the importance of combining tolerance to all three subsoil constraints into varieties intended for widespread adoption.

A Na exclusion trait was backcrossed into the background of the boron tolerant variety Kalka three times (BC_3), and a population of 196 F_2 derived lines selected. The trait segregated in ratios indicative of control by a single dominant gene at all stages of the backcross process, and full recovery of the Na excluding ability of the donor parent was

achieved. Na exclusion reduced the Na concentration in grain and it was found that grain analysis can be used as an accurate selection method.

The population was sown at three field sites with subsoil salinity levels in excess of 10dS/m ECe. A significant yield difference between Na excluding and non-excluding lines was detected at one of these sites; however, one site suffered from a severe infection of crown rot (*Fusarium pseudograminearum*), while the other has been shown to be dominated by high pH. In the latter site, the sensitivity of the recurrent parent Kalka to high pH restricted root growth to the upper soil layer, above that affected by transient salinity.

Na^+ exclusion was expected to increase the concentration of K^+ in plant tissue, but it was found that other ions were also affected. The cations Ca^{2+} and Mg^{2+} increased in concentration, while the concentration of the anions Cl^- and SO_4^{2-} decreased, suggesting that the exclusion of Na^+ ions was to some degree balanced by changes in concentration of other ions. The specific ions affected and the relative change in each varied depending on the soil. The increase in concentration of the low Na accumulating genotypes was large enough to be the difference between deficient and adequate nutrition of K, Mg and Ca at some field sites.

Bread wheat is able to exclude Na, so whole plant concentrations are commonly 5-10 fold lower than that of durum; however the variety Krichauff was shown to exclude more Na than its sister variety, Worrakatta. A population of F_5 derived lines developed from the cross between these two varieties, was yield tested at four sites affected by transient salinity. The population was sampled at the Redhill site, and assessed for Na concentration

in the whole tiller by inductively couped plasma (ICP) spectrometry. Significant correlations were detected between the Na concentration at Redhill and grain yield at Buckleboo, and screenings (%) at Port Pirie, indicating that the Na exclusion of Krichauff can have a beneficial effect on transient salinity tolerance.

Bulked segregant analysis revealed that the Na exclusion trait of Krichauff was controlled by a QTL on the long arm of chromosome 4B, which was linked to the microsatellite marker *gwm149*. This marker explained 61% of the variation in Na concentration among the lines included in the bulks.

A synthetic hexaploid (*Triticum aestivum*), which was identified as having the ability to tolerate high internal concentrations of Na, was able to maintain a significantly higher green leaf area than either Kharchia 65 or Krichauff when irrigated with saline nutrient solution. A significant salinity x genotype interaction occurred, which resulted in the synthetic line having higher fresh shoot weight in 75mmol/L NaCl. ICP analysis revealed that there was no significant difference in Na concentration, suggesting that an internal tissue tolerance mechanism, rather than exclusion was responsible for the observed tolerance.

This work has been incorporated into local breeding programs and has illuminated agronomic work on toxic subsoils and nutrient deficiencies.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Chapter 1.

GENERAL INTRODUCTION

Transient salinity has only been recognised as a problem of field crop production in recent years (Rengasamy 2000, 2001). Consequently, there has been no study published which has adequately investigated the effects of transient salinity on crop growth. The impact of salinity on field crops has been studied, but not in soils affected by transient salinity. For example, work by Ayres (1952) pertains to North American irrigated systems, while Bole and Wells (1979) and Richards (1983) studied the effects of irrigation induced salinity on crop growth.

Transient salinity, unlike dryland (seepage) salinity is not associated with a shallow water table. Affected soils typically contain high concentrations of soluble salts in the subsoil, but this varies with depth and changes throughout the season in response to rainfall, surface evaporation and the usage of water by vegetation (Rengasamy, 2001). After rainfall, soil water is generally readily available to plants and the salt is diluted enough to have little adverse effect on crop growth. However, as the soil dries the roots extract more water from further down the profile. As water is removed from the profile and saline water moves up from deeper soil layers by capillary action, salt concentration increases in the root zone. This most commonly occurs during grain fill when the frequency of rainfall events is diminishing and the water requirement of the crop is increasing (Rengasamy, 2001).

The mapping work of David Maschmedt (PIRSA Land Information, 2001) provides an indication of the magnitude of the problem in South Australia. If a subsoil salinity level of

4 dS/m (ECe) is considered to affect crop growth (Maas and Hoffman, 1977), the total cereal cropping area affected is probably in excess of sixty percent.

Transient salinity tends to have a non-uniform distribution within paddocks, making farming to land capability very difficult. Also, the marginal nature of cropping in the affected regions, means that amelioration and management options are severely limited by cost (Kennewell, 1999). Consequently, plant breeding is likely to be the most suitable option for improving production on affected areas (Armstrong *et al.*, 2001; Holloway *et al.*, 1992; Rengasamy, 2001). Rengasamy cites the success of breeding for boron tolerance in South Australia as an example of the progress that can be achieved in breeding for tolerance to subsoil constraints.

Commercial durum varieties have been shown repeatedly to be more intolerant of salinity than bread wheat (Francois *et al.* 1986; Rawson *et al.* 1988) and this intolerance is reflected in the distribution of durum production in South Australia. The current major areas of durum production are the Adelaide Plains, northern Mt. Lofty Ranges and York Peninsula, which are dominated by subsoils with less than 4 dS/m ECe. Other major cropping regions that could be suitable for producing high protein durum, such as the Eyre Peninsula, Murray Mallee and the coastal plains stretching from Two Wells through Brinkworth to Port Pirie, are dominated by subsoils with salt concentrations of 4dS/m and above.

Transient salinity affects the growth of cereals in two ways. The first is the osmotic effect on water availability, while the second is the toxic effects of Na and Cl ion accumulation in the shoot of the plant (Munns *et al.*, 1995). Chloride toxicity is a more widespread problem

than sensitivity to Na, except among the graminaceous species, where Na is the dominant factor affecting plant growth in saline environments (Marschner, 1988). Consequently, genotypes that are able to exclude Na from the shoot are likely to more tolerant than other genotypes.

Munns *et al.* (2000) proposed that the ability of bread wheat to tolerate higher concentrations of salt than durum was due to its ability to exclude more Na from the plant shoot. This difference results in durum commonly accumulating five to ten times as much Na under the same conditions. However, Munns *et al.* (2000) have identified three landrace accessions with Na exclusion comparable with bread wheat. Among this group, Na49 had the highest level of exclusion and will be used as a donor parent in a backcrossing program to introgress the Na exclusion trait into the locally adapted, boron tolerant variety, Kalka. The material developed in this crossing program will be used to assess the value of the Na exclusion trait in the field.

The Na excluding ability of bread wheat is far greater than that of durum, yet the widely adapted variety Krichauff appears to exclude more Na than other bread wheat varieties, including its sister variety, Worrakatta (Liu, unpublished). Worrakatta also produces grain with a higher percentage of screenings than Krichauff at many locations in South Australia (South Australian Research and Development Institute, 1996; 1997; 1998; 1999; 2000). A population will be developed from the cross between these two varieties, to investigate if a significant difference in Na exclusion does exist between them and whether this is responsible for the larger grain size of Krichauff.

The principal aims of this research project can be summarised as follows:

- Characterise and assess the impact of transient salinity and other subsoil constraints on the growth of durum wheat in field experiments.
- Develop screening methods that accurately assess the tolerance to Na^+ of large numbers of segregants accurately, rapidly and cheaply.
- Introgress genetic material conferring tolerance to transient salinity into locally adapted material and assess the direct benefit of Na exclusion on grain production in the field.
- Analyse the genetic control of the Na exclusion traits in durum and bread wheats and identify genetic variation suitable for breeding programs.
- Analyse the relative concentrations of other plant nutrients in durum lines contrasting for Na uptake.

Chapter 2.

REVIEW OF LITERATURE

2.1 Salts in the southern Australian landscape

2.1.1 Origin of salts

The soluble salts found in the soils of the southern Australian cereal zone have originated from deposition on the soil surface by wind and rain and from the weathering of parent rock (Rengasamy, 2000, 2002).

Gunn and Richardson (1979) proposed that rain and dry fallout of marine origin (at current rates) account for the occurrence of salt affected land in coastal areas of eastern Australia, but that this was unlikely to account for a large proportion of salts further inland. Fallout further inland is much more likely to originate as dust from soils and playa lakes being blown into the air and carried in the direction of the prevailing wind (Gunn and Richardson, 1979). In supporting this theory, Gunn and Richardson (1979) refer to a study of rainwater chemistry in Victoria by Hutton and Leslie (1958), which found that at about 125km inland the origin of salts probably shifts from marine to terrestrial. Gunn and Richardson (1979) also cite work by Hingston and Gailitis (1976), who concluded that salts at coastal sites in Western Australia were dominated by those of marine origin, while at sites further inland salts from terrestrial sources were at least equal in quantity.

During the Pliocene, a lake, or series of lakes, known as Lake Bungunnia, spread over the western half of the Murray Basin. A clay sediment was deposited, now known as Blanchetown clay. From this deposit, the heavier mallee soils have since developed (McCord 1995).

During the late Pleistocene the lake drained as the sea receded and there followed two periods of extensive coastal dune formation (McCord 1995). During these periods, strong southwesterly winds covered the landscape with a blanket of fine calcareous loess containing high levels of boron and sodium salt. The soils that developed from this aeolean deposit, known as the Woorinen Formation, are characterised by high levels of $CaCO_3$ concentrated in the B horizon and, where the Blanchetown clay is close to the surface, high levels of Na^+ and boron (McCord 1995; Rathjen *et. al.* 1999). Support for the theory that movement of material inland by wind has occurred is provided by Bowler (1976), who claims features such as lunettes, relict dunes, sand drifts and the infilling of old river lines provide evidence that periods of windy conditions occurred in the past.

The Blanchetown clay underlying the Woorinen Formation would have become more impervious over time through the leaching of salt. During the leaching process Na^+ ions are adsorbed onto clay particles, resulting in the clay layer becoming sodic and comprehensively impervious to future leaching. Consequently, salts and boron have been retained in the surface layers (Rengasamy, 2002).

On the upper Eyre Peninsula the dominant soils are of the Wiabuna Formation, which is thought to be equivalent to the Woorinen Formation of the Murray Basin (Wetherby pers. comm.). The Wiabuna Formation is considered to exist in two phases, the reddish phase, found at Penong, Minnipa, Wudinna and Buckleboo, and the calcareous phase of Cungena and Nunjikompita (K.G. Wetherby pers. comm.).

2.1.2 Composition of Salts.

Sodium salts (predominantly sodium chloride) dominate Australian soils (Rengasamy, 2002; Gunn and Richardson, 1979). Kennewell (1999) investigated paired soil pits (on and adjacent to a bare patch) at 20 sites in the northern agricultural area and on the upper Eyre Peninsula. The distinguishing feature of pits in the bare areas was higher salt concentration, with the dominant salt being NaCl. This is consistent with sampling by Holloway (1991) of the subsoil at Minnipa, also on the Eyre Peninsula. Sodium salts accounted for 82% of salts present, while 73% was NaCl (excluding carbonates and bicarbonates).

The dominance of sodium salts is not only restricted to Australia. Harmati (2000) sampled the three dominant salinised soil types along the Danube valley in Hungary and found that sodium salts accounted for 60 to 90% of the salts present in the three soil types. Similarly, Szabolcs (1989) states that the composition of salts varies depending on geology and climate, but that soils affected by sodium salts (Solonetz) are found in the Ukraine, Russia, Kazakhstan, Hungary, Bulgaria, Romania, China, USA, India, South Africa, and Australia, while calcium dominated soils (Gypsisols and Calcisols) are largely restricted to Mesopotamia, desert areas of the Middle East, the Central Asian Republics and some parts of Australia.

2.2 Forms of salinity

Salinity affecting dryland farming in southern Australia can be separated into two main types. Seepage salinity, associated with a water table at or near the surface, and dry saline land not associated with a water table. Kennewell (1999) refers to Herriot (1942) in claiming that agricultural scientists have been aware of dry saline land for more than fifty years. Despite this

knowledge, very little progress has been made in developing sustainable management of the problem (Kennewell, 1999).

A further distinction within the dry saline land classification can be made between 'magnesia' patches (Kennewell, 1999) and transient salinity (Rengasamy, 2002). These distinct forms of salinity originate from different processes and affect crop growth in different ways.

2.2.1 Salinity resulting from shallow water tables (seepage salinity)

Seepage salinity, or dryland salinity, is the obvious visual scalding associated with rising water tables. Although seepage salinity is a problem of increasing severity in some parts of Australia (particularly Western Australia), its occurrence in South Australia is of limited importance, despite a public perception of severity due to visual surface scalding. Because of the severe effects on plant growth in the scalded area and the lack of obvious salinity damage away from the actual scald, even large changes in salt tolerance of crop plants are unlikely to have a significant impact on farm profitability (Richards, 1983b).

2.2.2 Transient salinity

Transient salinity is not associated with a shallow water table and is not a problem that is rapidly increasing in severity. Affected soils typically contain high concentrations of soluble salts in the subsoil, but their effect on plant growth varies with depth and changes throughout the season in response to rainfall, surface evaporation and the usage of water by vegetation (Rengasamy, 2002).

A large proportion of South Australian subsoils are sodic and consequently, have very limited permeability to water (Rengasamy, 2002). Further, about half of South Australia's wheat growing area receives less than 350mm rainfall annually (Black, 1998). The combination of sodic subsoils and low rainfall greatly reduces leaching of salt out of the root zone of annual crops (Rengasamy, 2002).

After rainfall, soil water is generally readily available to plants and the salt is dilute enough to have little adverse effect on crop growth. As the soil dries in spring and the water requirement of the crop increases, the roots extract water from further down the profile. As water is removed from the profile, saline water moves up from deeper soil layers by capillary action, increasing salt concentration in the root zone. Increases in salt concentration in the soil solution affect the crop during dry periods throughout the growing season, but most commonly this occurs during grain fill when the frequency of rainfall events is diminishing and the water requirement of the crop is increasing. Salt levels over 4dS/m EC_e have both a toxic effect on plants, as well as an osmotic effect reducing the availability of soil water (Rengasamy, 2002).

2.2.3 Magnesia patches

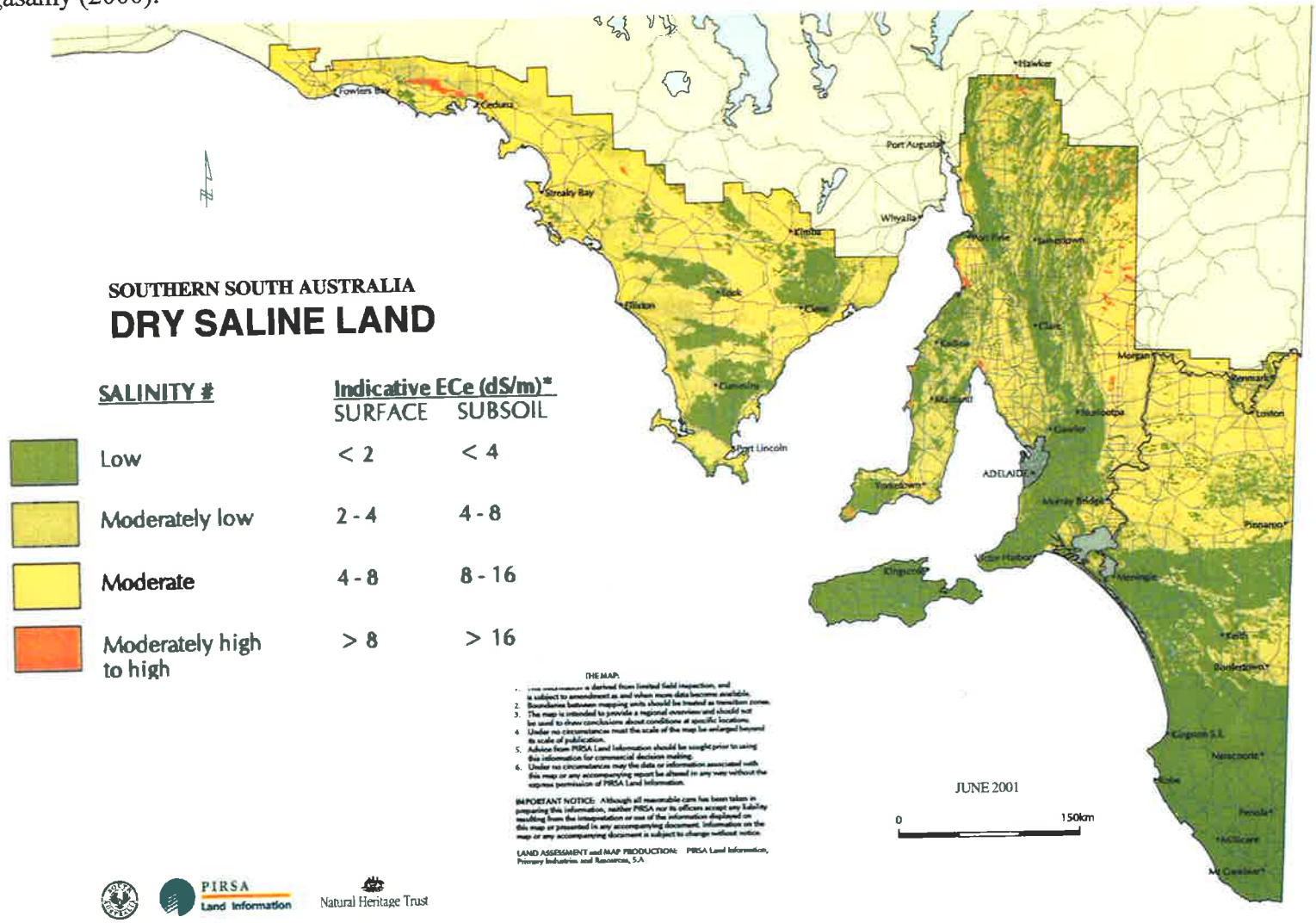
Magnesia patches occur on soils affected by transient salinity where the surface soil is salinised, causing an inhibition of germination. Kennewell (1999) reported that an estimated 45,000 ha of South Australia was affected by magnesia patches. This is comparable with Hughes and Jeffery (1994), who claim 35,000 ha to be affected by dry saline land, with most of this being restricted to the upper Eyre Peninsula. This estimate refers to 'magnesia' country, or bare patches due to high surface salinity levels. These figures do not include saline subsoils, which are common across Eyre Peninsula (Hughes and Jeffery 1994).

The transient saline soils that are affected by magnesia patches tend to have loamy to clay-loam surfaces, which allow soil water to move to the surface by capillary action and evaporate at the surface. Duplex soils with sandy A horizons are not subject to the same process due to the larger size of soil pores. Consequently salts tend to accumulate at the top of the B horizon, where they have less effect on germination (Rengasamy pers comm.).

2.3 The distribution of transient salinity in the South Australian cereal growing regions

An estimate of the area affected by transient salinity can be drawn from the mapping work led by David Maschmedt (PIRSA Land Information, 2001) (Figure 2.1). If a subsoil salinity level (ECe) of 4 dS/m is considered to affect crop growth (Maas and Hoffman, 1977), about one third of the mapped area (the agricultural districts of S.A.) is affected by transient salinity; however, much of the unaffected area is accounted for by the Adelaide Hills, lower South-east and Kangaroo Island, all of which is predominantly utilized for horticulture, urban development or the grazing of livestock. Also, large areas of Eyre Peninsula that are not affected by transient salinity are not cropped due to the predominance of sheet calcrete (A. Rathjen and D. Maschmedt, pers. comm.). When these areas are taken into account, the actual area is probably above sixty percent of the total cereal cropping area.

Figure 2.1. Distribution of areas affected by transient salinity (dry saline land) in the agricultural regions of South Australia. Reproduced from PIRSA Land Information (2001). Dry saline land is a term used by PIRSA to describe the transient salinity of Rengasamy (2000).



2.4 The effects of salinity on plant growth

By definition, saline soils contain soluble salts in quantities that adversely affect plant growth, but which have a sodium adsorption ratio (SAR) of less than 15 (Maas and Grieve, 1987).

The effects of salinity on the growth of cereals has been investigated and reported by many researchers. Inhibitory effects have been shown for all stages of plant development, from germination through to grain fill. The inhibitory effects of salt on germination and emergence are well documented (Sayad, 1985; Prakash and Sastry; 1992; Boubaker, 1996; Rawson et al., 1998; Epstein et al., 1980; Schaller et al., 1981). Studies on the effect of salinity at later stages of growth have demonstrated reduced water uptake and transpiration, tillering, shoot weight, rooting depth and grain yield (Holloway, 1991; Francois et al., 1986; Maas and Grieve, 1988; Shalhevit and Bernstein, 1968; Cerda and Bingham, 1978; Aceves et al., 1975; Holloway and Alston, 1992; Maas and Grieve, 1990; Ayers et al., 1952). Francois *et al.* (1986) reported a significant shift to earlier maturity in response to salinity.

The significant reduction in rooting depth (Holloway, 1991; Holloway and Alston, 1992) has important implications for water use efficiency for dry land farming in southern Australia, as the ability to use subsoil water is closely related to rooting depth (Hamblin and Tennant, 1987). Subsoil water and nutrients are much more important in dry land cropping systems than in irrigated soils, due to the reliance on subsoil uptake as the topsoil dries (Rengasamy, 2000).

Multiple problems occur in many soils where salts have accumulated. These soils also tend to contain toxic levels of boron and carbonates (Cartwright *et. al.*, 1986; McCord, 1995; Rathjen

et al., 1999; Rengasamy et al., 1992). Research by Nuttal (in Armstrong et al., 2001) revealed that a significant interaction occurs between salt and boron and that soil salinities commonly found in subsoils reduce the critical concentration of boron by at least 3-fold in a glasshouse assay; however, similar experiments of Bingham *et al.* (1987) failed to detect a significant interaction.

The causes of inhibition of plant growth by NaCl are many and varied, but are likely to result from either an inability to regulate ionic and osmotic environments (Reid and Smith, 2000).

2.4.1 Toxic effects on plant metabolism

Ion excess

As discussed in Section 2.1.2, the predominant salt in the subsoil is NaCl, so it is not surprising that salt toxicity in the field primarily results from the excessive accumulation of Na^+ and/or Cl^- in the shoots.

Evidence that reductions in plant growth in response to salinity result from ion excess, rather than simply an osmotically induced water deficit, have been shown repeatedly by comparing plant growth in isosmotic solutions of salt and polyethylene glycol (PEG). For example, the growth of beans (Lagerwerff, *et al.*, 1961), maize (Gollek, *et al.*, 1973) and barley (Storey and Wyn Jones, 1978) was significantly better in PEG than it was in salt solution in three separate studies. This is convincing evidence, because PEG can be taken up by the plant and have its own detrimental effects on plant growth (Greenway and Munns, 1980).

Na/Ca interactions

Reductions in plant growth and increased Na^+ accumulation under saline conditions have been observed at low Ca^{2+} concentrations (Wadleigh and Bower, 1950; Wieneke and Läuchli, 1980). It is proposed that the reduction in growth results from an increase in membrane permeability, which appears to be due to a high Na/Ca ratio, as the same concentrations of Ca^{2+} in the absence of salt does not affect growth (Greenway and Munns, 1980). Reid and Smith (2000) concluded that the Ca^+ dependent component of the response to salinity appears to involve several different Ca^+ related processes, some of which are only indirectly affected by sodium. They refer to the following examples in the literature,

- suppression of Ca activity by Na (Cramer and Lauchli, 1986),
- general stabilization of membrane structures by Ca (Pooviah and Reddy, 1987),
- improved ion channel discrimination against Na (Schroeder and Hagiwara, 1989; Lauchli, 1990),
- electrostatic interactions of Na and Ca in the cell wall (Stassart et al., 1981) and cell membranes (Cramar et al., 1985; Lynch et al., 1998; Kinraide, 1994; Kinraide, 1999; Yermiyahu et al., 1994), and
- inhibition of Ca uptake and xylem transport by Na (Lynch and Lauchli, 1985).

The Na/Ca interaction has been believed to be more of a confounding factor in solution culture experiments, rather than an interaction observed in the field. This was because the interaction only occurs in saline soils that are high in CO_3^{2-} (carbonate) as well as Cl^- and therefore was ecologically irrelevant (Greenway and Munns, 1980); however, work on the alkaline sodic soils affected by transient salinity (Holloway, 1991; Rengasamy, 2002) in South Australia

provides evidence of sodium carbonate/bicarbonate ions and toxic levels of Na^+ and Cl^- occurring in the same subsoil.

Munns (1993) argues that salts taken up do not directly control plant growth by affecting turgor, photosynthesis, or the activity of any one enzyme; rather, the accumulation of salt in the older leaves by transpiration hastens their death. This in turn reduces the supply of assimilate and hormones for the growing points.

2.4.2 Osmotic effects of salt on plant growth

Although there is evidence of amelioration of Na toxicity by Ca over a range of salt concentrations, there is a Ca independent component of Na inhibition of growth that increases with increasing salt concentration (Maas and Grieve, 1987). At these higher levels of salt, a large proportion of the inhibition of plant growth is probably accounted for by osmotic effects of the growth media (Munns et al., 1982; Bliss et al., 1986).

Rengasamy (Rengasamy, 2000, 2002; Rengasamy et al., 1992) describes the osmotic effect of increasing concentration in the soil solution. Figure 2.2 illustrates this effect in a red brown earth (alfisol) at an average root zone salinity of 4 dS/m. At this level of salinity, the osmotic effect, in addition to the matric potential of the soil, is large enough to shift the water potential (matric + osmotic) of the soil below the plant available limit of –1500kPa at a depth of approximately 23cm. This effect is of far greater importance in non-irrigated cropping soils, because as the soil dries during grain fill, the concentration of salt in the remaining soil water increases. This is illustrated in Table 2.1, which compares the change in osmotic potential with the change in soil water content of two soils with an $EC_{1:5}$ of 0.64dS/m.

Figure 2.2. Gravimetric water content and soil water potential (Matric and total) of an alfisol (red-brown earth) profile with an average root zone conductivity of 4dS/m ECe. Reproduced with permission from Rengasamy (2000).

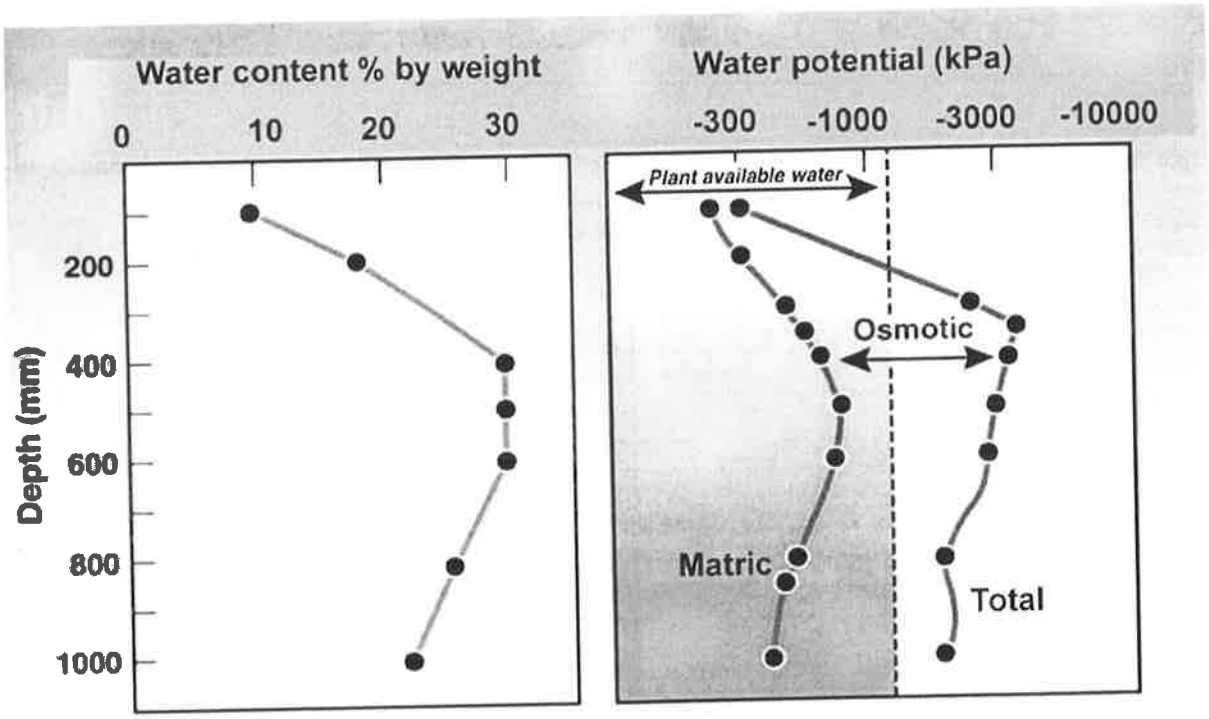


Table 2.1. Matric, osmotic and total water potential of an alfisol (loam) and a vertisol (clay). Reproduced with permission from Rengasamy *et al.*, 2003.

Soil water status	Water content ($g\ g^{-1}$)	ECc (dS/m)	Soil water potential (kPa)		
			Matric	Osmotic	Total
Alfisol					
1:5 soil:water	5.00	0.64	+0.1	-23.0	-22.9
Saturation	0.35	9.14	-0.1	-329.1	-329.2
Field capacity (FC)	0.20	16.00	-10.1	-576.0	-586.0
Drier than FC	0.14	22.86	-680.0	-823.0	-1503.0
Permanent wilting point	0.05	64.00	-1500.0	-2304.0	-3804.0
Vertisol					
1:5 soil:water	5.00	0.64	+0.1	-23.0	-22.9
Saturation	0.80	4.00	-0.1	-150.0	-150.1
Field capacity (FC)	0.40	8.00	-10.0	-288.0	-298.0
Drier than FC	0.16	20.00	-780.0	-720.0	-1500.0
Permanent wilting point	0.08	40.00	-1500.0	-1440.0	-2940.0

2.4.3 Two-phase response to salinity

Munns *et al.* (1995) used various wheat, barley and triticale genotypes to test the hypothesis that the growth response to salinity has two phases. In the first phase there was a large decrease in growth rate caused by the salt outside of the roots (response to osmotic potential), while in the second phase there was an additional decline in growth caused by salt toxicity within the plant (ion excess).

Genotypes that differed greatly in salt tolerance were grown in 0 or 150 mM NaCl solution culture for up to 6 weeks. After 4 weeks all genotypes showed a similar reduction in growth in

salt compared to the control. After this initial period the more sensitive genotypes showed a greater reduction in growth, which occurred after 60% of the leaves were dead (Munns et al., 1995).

These data strongly support the hypothesis of a two-phase response and therefore indicate that any genotypic differences in the first phase relate to the osmotic effect and not to a salt specific effect. There was very little genotypic variation for tolerance to osmotic stress amongst the genotypes tested. The second phase began only after toxic levels had accumulated in the leaves in adequate quantities to cause leaf necrosis and therefore a resultant reduction in available assimilate (Munns et al., 1995).

Munns *et al.* (1995) concluded that the length of the first phase was dependent on the concentration of salt in the soil, transpiration rate and the ability of the genotype to exclude Na. They also refer to the results of other researchers that indicate that the initial response to salinity is dependant on water potential, rather than the specific salt, as shown by the use of other osmotica (Delane et al., 1982; Cramer and Bowman, 1991; Yeo et al., 1991; Neumann, 1993).

Further support for the two-phase theory is provided by the results obtained by Ayres *et al.* (1952). Four barley and two wheat varieties were subjected to various saline irrigation treatments in field plots. No significant genetic variation was measured at germination and the early stages of development, despite a significant delay in emergence. The second phase of saline irrigation resulted in a level of yield reduction that was greatly affected by the stage of plant development when saline irrigation began. Plots that received the high salt treatment at

an early stage suffered the greatest level of damage, presumably due to a greater ionic accumulation over time, while a significant interaction between genotype and salt treatment affected grain yield in this second phase.

2.5 Options for improving cereal production on land affected by transient salinity

Transient salinity is a widespread problem, particularly on the Eyre Peninsula and in the Murray Mallee. The distribution within a paddock tends to be non-uniform, making it a difficult problem to manage. Also, the marginal nature of cropping in the affected regions means that amelioration or management options are severely limited by cost/ha (Kennewell, 1999).

Allowing the surface of transient saline soils to become bare exacerbates magnesia patches, increasing surface evaporation and thereby increasing salt concentration at the surface. Trials conducted on bare patches in 1993 illustrated that the severity of patches could be reduced by placing a mulch layer of either 25mm of sand, or 8t/ha of cereal straw on the soil surface, so as to reduce evaporation (Kennewell, 1999). If rainfall is greater than evaporation, the salts will be flushed from the surface soil, allowing improved germination. While the application of mulch to affected soils in the marginal agricultural districts is unlikely to be economically feasible, the results of this research did provide evidence that retaining crop residues has a beneficial effect.

Ameliorating magnesia patches will allow a crop to germinate and emerge, but the salt will still be present further down the profile. Depending on seasonal conditions, this salt will then affect the crop as transient salinity.

Armstrong *et al.* (2001) claims that there is a growing awareness of the subsoil constraints affecting crop growth and water use efficiency in southern Australia, and that overcoming these limitations will produce a quantum leap in productivity. They believe that plant breeding offers the best long-term strategy for overcoming these constraints, primarily because it is highly unlikely that amelioration is a practical or cost effective option. Rengasamy (2002) also believes that breeding for tolerance to transient salinity will help to alleviate the problem, and states that the success of breeding for boron tolerance in South Australia serves as an example of the progress that can be achieved. The suggestion that breeding for tolerance to transient salinity is a valid option is further supported by Holloway and Alston (1992), who claim that using cultivars with a high degree of salt and boron tolerance will lead to an improvement in wheat production on Upper Eyre Peninsula and in other regions with similar constraints.

2.6 Adaptive mechanisms for tolerance to transient salinity

Two basic mechanisms for salt tolerance in plants are documented. Reduced transport of salt to the shoots, and tolerance to high leaf salt concentrations by sequestration within cell vacuoles (Greenway and Munns 1980); however, there is possibly also a role for the production of neutral organic solutes in the cytoplasm.

2.6.1 Ion Exclusion

Na and Cl accumulate in the leaves of cereals as a result of transpirational flow from the roots. If the plant has no mechanism to exclude entry into the shoot, the concentrations of these ions will reach toxic levels, inhibiting metabolic enzymes and eventually result in necrosis of the older leaves (Greenway and Munns 1980).

The relative importance of Na and Cl toxicity varies between plant species and consequently, so will the relative value of breeding for exclusion of one or the other. Marschner (1988) claims that in general, sensitivity to chloride toxicity is more widespread than sensitivity to Na. This is particularly true in the woody perennial plants, such as avocado (Bingham, *et al.*, 1968), conifers (Alt *et al.*, 1982), citrus (Maas, 1993), grapes (Alexander and Woodham, 1968; Downton, 1977; Ehlig, 1960; Woodham, 1956), stone fruits and almonds (Bernstein *et al.*, 1956); however, Chavan and Karadge (1980) also found this to be the case in peanut.

In the graminaceous species, such wheat (Gorham, 1993) and rice (Flowers *et al.*, 1991; Yeo, 1993) sensitivity to Na appears to be the dominant factor restricting plant growth in saline environments. This is also indicated in the fact that little genetic variation was found within the *Triticum* species for Cl exclusion (Gorham *et al.*, 1990), suggesting that Cl accumulation has not been a factor that has affected the evolution of these species. Contrary to this theory, Chauhan and Chauhan (1984) found that substrate Cl did restrict the growth of wheat, but this did not occur below a plant tissue concentration of 0.2%, or 564mmol/kg dry weight. This is above the critical value of 348mmol/kg of Na (Weir and Cresswell, 1984).

Clearly, wheat genotypes that are able to exclude Na from the roots are likely to have more tolerance to Na than other genotypes. Evidence of this can be found in the observation that many South Australian varieties accumulate less Na when compared to other genotypes. Varieties such as 'Krichauff', 'Halberd', and 'Machete' have an ability to exclude Na to a level that is at least equivalent to the tolerant variety 'Kharchia' from India, which has been used widely in physiological experiments (C-Y Liu unpublished). A survey of the trial sites used by the University of Adelaide wheat breeding programs in 2000 indicated that about half

of these sites are moderately saline, having subsoil salinity levels between 8-12 dS/m (A. Rathjen unpublished data). Presumably, inadvertent selection for salt tolerance at breeding sites such as these has been great enough to select a predominance of genotypes which exclude more Na from the shoot than is observed in introduced varieties.

Commercial durum varieties have been shown repeatedly to be more intolerant of salinity than bread wheat (Francois *et al.* 1986; Rawson *et al.* 1988). Munns *et al.* (2000) proposed that this difference in tolerance was due to the greater ability of bread wheat, compared to durum, to exclude Na from the plant shoot.

Many authors have proposed reduced Na accumulation in the plant shoot as a mechanism of tolerance (Flowers *et al.* 1977; Greenway and Munns 1980; Chhipa and Lal, 1995), while Maathuis and Prins (1990) reported that salt tolerant *Plantago maritima* accumulated lower concentrations of Na than the intolerant species *Plantago medica.* In an attempt to identify *Triticum turgidum* with enhanced Na exclusion, Munns *et al.* (2000) screened fifty-four genotypes including accessions of the *T. turgidum* subspecies, *durum, turgidum, polonicum, turanicum and carthlicum.* This work identified the durum accession Na49 as having a high level of Na exclusion and it was proposed that this line could be used as a donor parent in a backcrossing program for improving the salt tolerance of commercial durum varieties.

Dvorak *et al.* (1994) attempted to improve the Na exclusion of durum wheat by transferring the *Kna1* locus of bread wheat into a durum background by homoeologous recombination of the long arm of chromosome 4D with 4B of the durum variety 'Langdon'. A series of 4D recombinants were produced; nine carrying *Kna1* and fifteen without. When the lines where

grown under saline conditions the recombinants with *Kna1* had significantly more biomass, but no significant difference in grain yield was measured.

2.6.2 Compartmentalisation of excess ions

Some genotypes are able to 'compartmentalise' ions into the vacuole and therefore reduce cytoplasmic levels such that they do not inhibit metabolic processes (Greenway and Munns 1980). Barley is a notable; although it is related to bread wheat, it often has leaf Na concentrations ten times greater than hexaploid wheat (Munns pers. comm.), while being at least as salt tolerant as wheat (Bole and Wells, 1979; Heakel *et al.*, 1981; Rawson *et al.*, 1988; Richards *et al.*, 1987). Presumably variation exists within hexaploid wheat and durum for compartmentalisation, or can be introgressed from close relatives.

2.6.3 Neutral cytoplasmic solutes

Neutral solutes such as proline (Story and Wyn Jones, 1977), glycinebetaine (Story and Wyn Jones, 1977) and sucrose (Gauch and Eaton, 1942) have been found to increase in concentration in the cytoplasm under saline conditions. These compounds potentially have two roles in salt tolerance. Firstly, plants that are able to sequester ions to the vacuole may exhibit osmotic regulation of the cytoplasm against high concentrations of Na^+ and Cl^- ions in the vacuole (Kramer et al., 1977). Secondly, there is some evidence that these solutes provide a protective role against inhibition of enzymes by elevated ion concentrations (Pollard and Wyn Jones, 1979; Greenway and Munns 1980).

2.7 Screening methods for the identification of salinity tolerance

The rate of progress in a breeding program for a subsoil toxicity is largely determined by the accuracy and duration of the screening technique. Where extensive field testing is required, the number of generations can be restricted (1 per year) and progress slow. For this reason a large amount of effort tends to be expended on the development of laboratory based techniques under controlled conditions (Rathjen et al., 1993; Chhipa and Lal, 1995). These techniques are generally based on the exacerbation of the physiological response to stress (Rathjen et al., 1993). Two examples of this are the screening for boron tolerance in soil (Paull, 1990) and solution culture (Campbell et al., 1998), and the observation of recovery of seedling roots after exposure to aluminium (Polle et al., 1998).

2.7.1 Screening for tolerance at germination and early stages of growth.

Tolerance screening of large numbers of genotypes on naturally saline soils is extremely difficult due to extreme spatial and temporal variation (Richards, 1983), and strongly affected by site x year interactions. For example, areas affected by transient salinity may actually be higher yielding in high rainfall years, because the salt is diluted, while nutrient concentrations will be higher due to less product removal in average years (Rathjen and Cooper, 2000).

To avoid inaccuracies arising from spatial variation in field trials, as well as the number of generations possible per year, screening for germination and seedling survival under extreme conditions in NaCl solution culture are often undertaken (Epstein et al., 1980). While variation

in tolerance has been demonstrated many times (Sayad, 1985; Sadat Noori and McNeilly, 2000; Hannon and Bradshaw, 1968; Ahmad and Wainwright, 1977; Lein et al., 1985; Ashraf et al., 1986; Azhar and McNielly, 1989; Prakash and Sastry, 1992; Boubaker, 1996; Schaller et al., 1981), response at early stages of development may bear no resemblance to tolerance ratings at later stages of development and under field conditions (Ayers et al., 1952). Rawson *et al.* (1988) expressed concern at the use of such screening methods because germination involves processes that are not found in established seedlings. Also, very young roots, which have not formed a casparian strip, cannot exclude NaCl like mature roots. Munns *et al.* (1995) support these concerns in their proposal of a two-phase model of response to salinity.

Schaller *et al.* (1981) did demonstrate a correlation between emergence of barley cultivars under saline conditions and performance through to grain yield in the field; however, it should be noted that this correlation is based on the results of one field site in one year, with no plot establishment data recorded. Despite the lack of establishment data, they did state that 'the different behaviour of the two groups (high and low germination in salt) does not appear to be attributable to differences in stand establishment'. This may be the case, but more rigorous and extensive field testing across sites and years would be more convincing. Schaller *et al.* (1981) did refer to Maddur (1976) who suggested an association between tolerance at germination and early growth, with more vigorous seedling establishment and subsequent growth. Supporting evidence is provided by the experiments of Rawson *et al.* (1988), which showed that intrinsic high growth rate did provide additional tolerance at later stages of development.

2.7.2 Screening at later stages of development

Screening of the whole growth cycle of wheat and other cereals in solution culture (Sayad, 1985; Munns et al., 2000) and soil based (Maschhady and Heakal, 1983, Heakal et al., 1981) experiments has been used extensively. Although longer in duration than methods used at germination, they are deemed to be better indicators of field response to salinity (Rawson et al., 1998).

Most of these methods involve growing plants in modifications of ½ strength Hoaglands solution, plus the salt treatment, plus 5mM supplemental calcium (Munns et al., 2000). This supplemental calcium is critical to overcome the effects of sodium induced deficiency by inhibition of Ca^{2+} uptake (Greenway and Munns, 1980; Reid and Smith, 2000). Munns (pers. comm.) believes that if the Ca content in the leaves of plants under salinity stress is equal to control plants, there is adequate Ca^{2+} in the nutrient solution.

Where the genotypes to be screened in this type of experiment have substantially different maturity times, total biomass relative to the control may be a better measure of tolerance than relative grain yield (Kingsbury and Epstein, 1984).

2.7.3 Screening in the field

Examples of the use of field trials to assess salt tolerance are less common in the literature. This is explained in part by the complexity of the environment compared to controlled environment experiments. Spatial variation within the paddock (Richards 1983; Jana, 1993), and the effects of site x year interactions (Rathjen and Cooper, 2000) make it very difficult to

obtain useful results. Despite this fact, Rathjen *et al.* (1993) believe that field research is an irreplaceable tool in plant breeding, not only for investigating known factors, but because it often opens up new areas of research by elucidating factors that have not previously been considered.

McColl (1987) and Richards (1983) both obtained significant genotype x salinity interactions from field experiments, however both recommended selection for tolerance to low to moderately saline soils take place on non-saline soils. The conclusions of Richards (1983) were based on the fact that salinity has a very patchy distribution, with most of the yield coming from the least affected areas of the paddock; however, this conclusion was derived from studying irrigation environments and assumes that the highest yielding genotypes are comparatively lower yielding on unaffected soil. It also assumes that selection for other traits conferring yield *per se* would not be selected on saline soils.

McColl (1987) showed by regression analysis of individual lines that although the slope of the response to increasing salinity varied between genotypes, the lines converged at the higher concentrations of salt. Consequently, the highest yielding lines in a low salt environment were also the highest yielding in most environments. It would therefore be easiest to select high yielding lines in low salt environments where the regression lines were more diverged. Jana (1993) points out that in both of these experiments, the cultivars studied were not specifically developed for their response to salt stress and only a limited number of genotypes were tested. Consequently, the question of what constitutes an optimal environment for selection in breeding for saline environments remains unresolved.

In studying the response of barley to boron toxicity in South Australian soils, Jefferies (2000) investigated the response of different genotypes to subsoil variation in boron concentration. Grain yield and the percentage of grain retained above a 2.5mm screen were measured as variables, while soil samples were taken after harvest from each of the check plots for boron analysis. The boron data was then used as a covariate in spatial analysis of the data to provide an estimate of the effect of boron on the response variables, grain yield and size. Regression analysis of data from Minnipa (boron toxic) on the genotype mean of Pinery and Horsham (non-toxic) was used to produce fitted values, which were subtracted from the observed to produce adjusted estimates of the genotype response to the subsoil toxicity (Jefferies, 2000).

The methods used by Jefferies (2000) at the Minnipa site provide an assessment of the effect of the soil covariate on grain yield; however, the use of two sites putatively unaffected by boron as the basis for an estimate of genotypic yielding ability, appears to be seriously flawed. No assessment of other yield limiting factors (biotic or abiotic) was undertaken at those sites, let alone an assessment of genotypic responses to those factors. Consequently, deviations from the line of regression between the boron toxic and non-toxic sites may be controlled by numerous unknown factors at the non-toxic locations.

The effects of transient salinity and boron is similar in terms of crop growth and interaction with seasonal rainfall events. Methods similar to those used by Jefferies (2000) at the Minnipa field site could equally be applied to investigations into the effect of variable transient salinity at individual sites.

2.8 The availability of genetic variation in durum wheat

The period of crop development when transient salinity is likely to affect crop growth is between anthesis and grain fill, when the soil profile is drying, temperatures and evaporation are increasing and the water requirement of the crop is at its highest. In this situation, genetic variation for salt tolerance during germination is of little value, because the mechanisms involved in conferring tolerance at germination are different to those in adult plants (Epstein, *et al.*, 1979; Munns *et al.*, 1995; Norlyn, 1980; Sayad, 1985). This means that the conditions of transient salinity restrict the choice of tolerance mechanisms to those which are expressed in adult plants, and more particularly, during anthesis and grain fill.

Of the three tolerance mechanisms discussed in Section 2.6 (Na exclusion, Na sequestration to vacuoles and the production of neutral cytoplasmic solutes), variation has only been discovered for Na exclusion within the species *Triticum durum* (Munns *et al.*, 2000). There have been three durum landrace accessions identified, which exhibited Na exclusion of a similar magnitude to the bread wheat variety 'Janz'. These lines will be studied in detail in this project, with the aim of transferring the Na exclusion trait into backgrounds adapted to local conditions.

Any further search for variation for either Na exclusion, or other tolerance mechanisms should start with the material best adapted to local commercial conditions, followed (stepwise) by material which is genetically more distant. This will maximise the chances of identifying the trait of interest in a closely related background, thereby enabling the trait to be transferred into locally adapted material using fewer cycles of backcrossing.

The first step is to screen all of the locally adapted varieties and breeding for tolerance to transient salinity. This approach was used successfully to search for genetic variation for boron tolerance in bread wheat, identifying the South Australian variety 'Halberd' as being moderately tolerant to boron toxicity (Paull, 1985). The discovery of halberd enabled boron tolerance to be rapidly introgressed into a wide range of material within the Waite Institute wheat breeding program, which had hitherto been dominated by intolerant genotypes and ultimately resulted in the release of the variety BT-Schomburgk. This variety incorporated the boron tolerance of Halberd in the background of Schomburgk and was the first variety specifically developed for boron tolerance (Rathjen *et al.* 1995). If variation for the trait of interest is not discovered in the locally developed material, the next step is to screen commercial genotypes from other environments, but preferentially from regions also affected by the constraint.

If variation cannot be identified in commercial varieties or breeding material, the next step is to screen a wide range of durum landraces held by the Australian Winter Cereals Collection, which was the method used by Munns *et al.* (2000) to identify the three Na excluding durum genotypes. The selection of landraces for screening could be done either randomly, or by targeting regions of origin known to be affected by similar conditions.

If suitable variation cannot be identified amongst durum landraces, the next step would be to use variation in other related species. The transfer of the *Kna1* locus from the D genome of bread wheat into durum by homoeologous recombination (Dvorak *et al.*, 1994) is an example of how such variation can be utilised.

Chapter 3.

AN INVESTIGATION INTO THE EFFECT OF SUBSOIL CONSTRAINTS AT SELECTED SITES WITHIN SOUTH AUSTRALIA

3.1 Introduction

Transient salinity has only been recognised as a problem of field crop production in recent years (Rengasamy 2000, 2002). Consequently, there has been very little published on the effects of transient salinity on crop growth, whereas, the impact of irrigation and seepage salinity on field crops has been extensively studied. For example, work by Ayres (1952), Bole and Wells (1979) and Richards (1983) studied the effects of irrigation induced salinity on crop growth.

South Australian soils affected by transient salinity have also accumulated high concentrations of boron and carbonates (McCord 1995; Rathjen *et al.* 2000; Rengasamy *et al.* 1992). The relative importance of these three limiting factors has not been defined, although the damage caused by boron toxicity is well documented (Cartwright *et al.* 1986; Paull *et al.* 1988) and breeding for boron tolerance has been successful (Rathjen *et al.* 1995).

Interactions between these three sub-soil factors are also possible. Nuttal (in Armstrong *et al.* 2001) found that commonly occurring levels of soil salinity significantly reduced the critical value for boron toxicity in a glasshouse assay.

3.2 Field experiments to investigate the effect of subsoil constraints on grain yield and screenings, 2001

3.2.1 Introduction

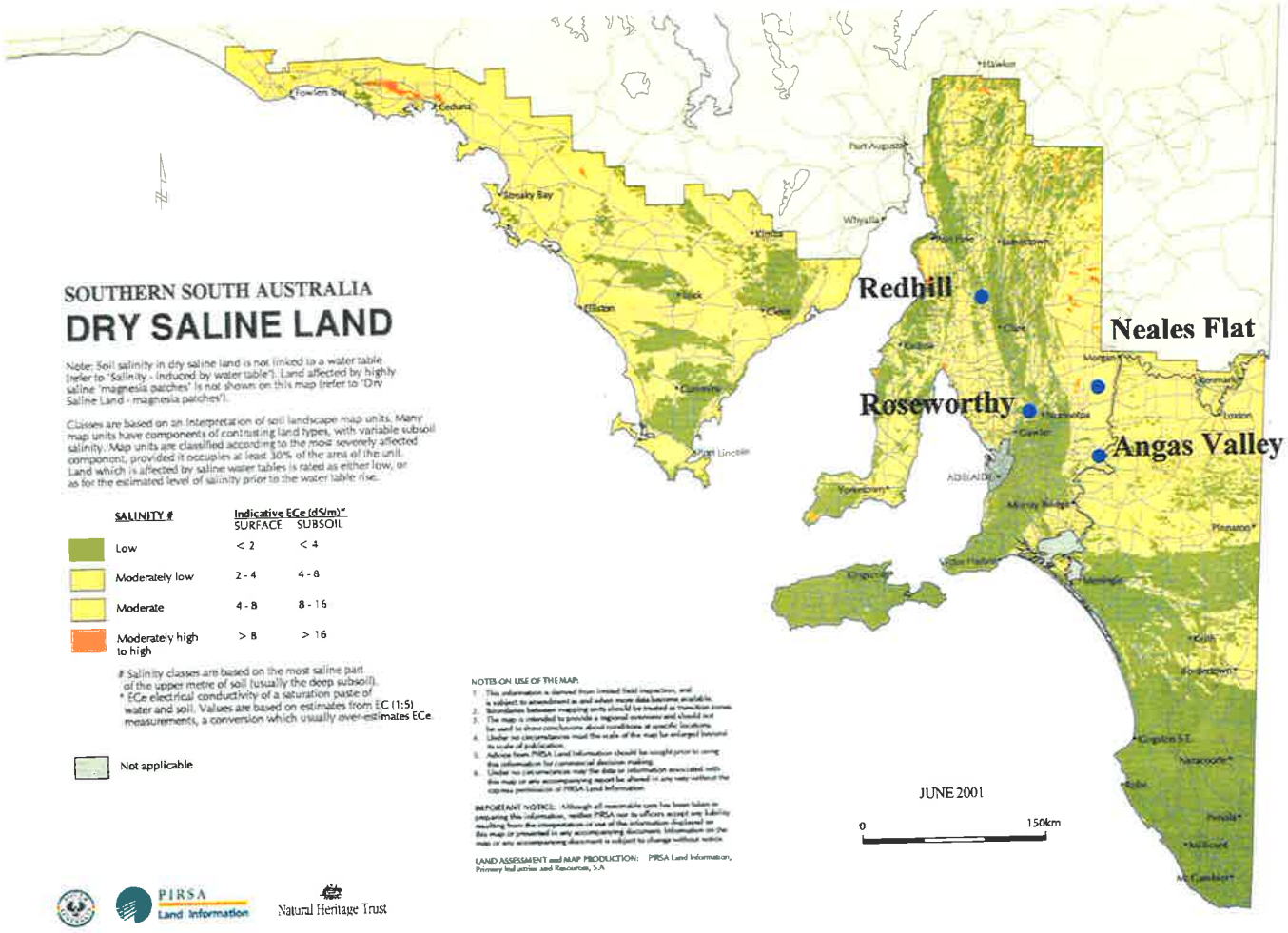
Field trials were sown on two sites at each of four locations known to be affected by transient salinity. At each location, the two sites were located in the same paddock, but on soils contrasting in salinity level, so any biological constraints and fertiliser history were much the same.

3.2.2 Materials and methods

The field experiments were conducted at four locations in South Australia that were affected by transient salinity, Redhill (RH), Roseworthy (RC), Neales Flat (NF) and Angas Valley (AV). The locations were selected initially by selecting geographically separated areas of the state that had a high proportion of subsoils affected by salinity levels of at least 4dS/m (ECe), according to the map produced by Maschmedt of PIRSA Land Information (2001) (Figure 3.1). Local farmers and agronomists were contacted so that they might suggest paddocks that had areas with salinity levels high enough to affect crop growth and which were suitable for experimental work.

The Redhill site was on the property of John and Ben Wheaton, who employ Mick Faulkner for agronomic advice. Faulkner had suspected salt to be responsible for the poor performance of durum crops on the paddock in which the trials were sown.

Figure 3.1. Distribution of areas affected by dry saline land in the agricultural regions of South Australia. The use of the term 'dry saline land' as used by PIRSA Land Information refers to transient salinity as described by Rengasamy, 2000. Reproduced from PIRSA Land Information (2001).



The Angas Valley site was on the property of Dr. Tony Rathjen, who had shown previously that there was variation for salinity in the paddock (unpublished), especially associated with the junction of the Woorinen and Blanchtown formations. The saline areas had been less productive historically than non-saline areas in years of low rainfall.

The Neales Flat site was selected by Mr. Nigel Steinborner of the University of Adelaide. Steinborner's father was a farmer on a neighboring property and was aware of the salinity problems present throughout the district and suggested that paddocks east of the Mount Lofty Ranges, on the property of Mr. Paul Schiller, were severely affected.

Unpublished data provided by Mr. Christopher Penfold of the University of Adelaide, indicated that a paddock of the Roseworthy Campus farm had subsoil salinity levels in excess of 6dS/m ECe associated with a drainage line. A neighboring paddock selected for this experiment had areas of similar salinity levels, as indicated by the EM38 conductivity meter.

At each of these four locations, trial sites were placed at both high (H) and low (L) salinity sites in the same paddock, so the total number of environments tested was eight (referred to as RHL, RHH, RCL, RCH, etc.). The within paddock site selection was performed using a Geonics EM38 ground conductivity meter to obtain an estimate of electrical conductivity. These measurements were verified by taking soil samples (0-20, 20-40 and 40-60cm) and measuring $EC_{1:5}$ in the laboratory.

The total number of entries was sixty-five, plus the durum variety Tamaroi, which was included every seventh plot as a check. The entries consisted of thirty-five bread wheat

genotypes, of which five were entered twice, while twenty-five durum genotypes were also included. All sixty-five entries were replicated in three complete randomized blocks, therefore the total number of plots (including thirty Tamaroi check plots) at each site was 225. Each of the eight field trials was sown in a rectangular array of 15 columns and 15 bays, so each replicate was contained within five bays.

The experiment was sown using the machinery and methods of the Waite wheat breeding program. The plots were sown using a modified 14 row drill so three plots, each four rows wide, were sown simultaneously. The inter-row spacing was 15 cm, while the plots were separated by one missing row, or 30 cm. The plots were arranged into 15 bays, each 6 m long, so that the total length of the experiment was 90 m. Thirty grams of bread wheat seed was sown over the full 6 m x 4 rows (60 kg/ha). The seeding rate was increased to 40g/plot (80kg/ha) for the durum entries, to allow for the high mean seed weight and poor tillering ability. Pathways of 1.8 m were sprayed with a knockdown herbicide between bays in the period between head emergence and anthesis to allow for automatic cleaning of the harvester. This reduced the harvested length of each plot to 4.2 m and the plot area to 2.52 m^2. Fertilizer (N:P:K:S 18:20:0:0) was applied with the seed, at a rate of 80kg/ha. Herbicides were applied when required in accordance with local practice.

The trials were harvested in December 2001 and the grain weighed. After harvest, the grain was cleaned using a Rationel Kornservice® SLN3 sample cleaner, which removed chaff and separated grain over a 2.2 mm screen. Grain weight below the screen was recorded and used to calculate the percentage of screenings in each sample.

Soil samples were extracted from all sites using the soil coring rig of the University of Adelaide, at the 0-10 cm, 30-40 cm and 50-60 cm profiles of every Tamaroi check plot. Electrical conductivity and pH were measured on 1:5 soil paste extracts from all samples, while extractable boron in hot $CaCl_2$ (Spouncer *et. al.*, 1992) was measured on the 30-40 cm soil samples only. The EC, pH and boron data were used as covariates in the statistical analysis to assess their impact on the variates grain yield and screenings. These covariates are refered to as EC(0-10), EC(30-40), EC(50-60), pH(0-10), pH(30-40), pH(50-60) and B(30-40).

Statistical analysis

Simple correlations were undertaken in Microsoft Excel 2000® to identify the soil covariates that were correlated with the variates, yield and screenings. Where more than one significant correlation was detected, multiple linear regression was undertaken (using Genstat 6®) to identify the soil covariates which explained the highest proportion of the variation. For example, it was likely that in many cases the $EC_{(1:5)}$ of the 30-40cm and 50-60cm samples would be highly correlated, hence only one of them could be regarded as contributing statistically to differences in yield or screenings. Consequently, yield could be correlated with $EC_{(1:5)}$ at 50-60cm, even if there was not appreciable root growth at that depth.

The soil covariates were only measured on the Tamaroi check plots. To obtain estimates on all plots, the level in each nearby check plot was weighted on the basis of distance between the plot being estimated and the check plot. This was performed using specialised plant breeding software, written within the University of Adelaide (Rathjen pers. comm.), which

has been used routinely for expressing the estimated yield of treatment plots relative to the check.

As only three replicates of each line were included in the design of the experiment, varieties were grouped into families based on common parentage to increase the number of observations for each 'genotype' (family). The estimated covariates were then plotted against the yield and screenings of these families of varieties to investigate whether genotypic differences in response were detectable.

3.2.3 Results

The Neales flat location was sown prior to the Schiller family making the decision to not sow the paddock due to continuing dry conditions. Hence, during grain fill, the experimental plots were the only wheat present in the paddock and extensive bird damage resulted in the experiment being unusable. Consequently, it was not harvested and soil samples were not taken.

Soil characteristics

The surface soils at all of these sites were clay loams, which meant that a conversion factor of eight could be used to estimate the conductivity of a saturated paste extract (ECe) salinity (Rengasamy pers comm.). On this basis, the salinity level that was likely to affect crop growth of 4dS/m ECe (Maas and Hoffman, 1977) would be approximately 0.5dS/m $EC_{1:5}$. All of the sites had topsoil salinity levels (0-10cm) which were unlikely to affect crop growth, but at the 30-40cm depth, the levels of the high salinity sites (AVH, RCH and RHH) at all three locations were sufficiently high to be expected to have an effect (Table 3.1). At the 50-60cm depth, the same three sites had mean salinity levels in excess of

0.5dS/m. The RCL site also had a conductivity in excess of 0.5dS/m EC1:5 at the 50-60cm depth.

High pH soils are very common across the South Australian cereal regions and this was reflected in the mean values measured. Soil pH levels (in water) greater than 8.5 are expected to have high concentrations of free $NaHCO_3$ and poor nutritional status (Peverill *et al.*, 1999). While the availability of the micronutrients, Zn and Mn are known to be reduced at high pH, no study has published a concentration curve for bicarbonate, or an actual critical value for pH, but Rengasamy (pers comm.) also accepts the value of 8.5 to be a point of reduced plant growth due to free bicarbonate. All of the sites had topsoil pH levels less than 8.5 in water, but greater than this level below 30cm (Table 3.1).

Peverill *et al.*(1999) state that soils with greater than 3mg/kg of boron present are likely to result in boron toxicity in cereal crops, although this is very dependent on the clay content of the soil. In sandy soils this concentration would be much more toxic than in clay soils. Four of the six sites (AVH, RCL, RCH and RHH) had a mean boron concentration greater than 3mg/kg at the 30-40cm sampling depth, while only the AVL and RHL sites were below this level (Table 3.1).

Table 3.1 Site mean $EC_{1:5}$ (dS/m) (measured at 0-10, 30-40 and 50-60cm intervals), pH (0-10, 30-40 and 50-60cm) and boron (mg/kg) (30-40cm) values for Tamaroi check plots. Levels of soil salinity >0.5dS/m $EC_{1:5}$ (Maas and Hoffman, 1977), pH >8.5 (Peverill *et al.* 1999) and boron >3mg/kg (Peverill *et al.* 1999) expected to restrict crop growth, have been highlighted.

Covariate	Sites					
	AVH	AVL	RCH	RCL	RHH	RHL
EC(0-10)	0.22 ±0.16	0.05 ±0.02	0.13 ±0.06	0.15 ±0.04	0.27 ±0.09	0.13 ±0.02
EC(30-40)	**0.71 ±0.15**	0.17 ±0.08	**0.62 ±0.41**	0.41 ±0.21	**1.08 ±0.18**	0.13 ±0.02
EC(50-60)	**0.81 ±0.09**	0.29 ±0.14	**0.95 ±0.51**	**0.72 ±0.25**	**1.56 ±0.34**	0.24 ±0.11
pH(0-10)	8.31 ±0.53	6.74 ±0.25	6.81 ±0.63	7.53 ±0.44	6.96 ±0.30	8.31 ±0.27
pH(30-40)	**9.81 ±0.11**	**8.66 ±0.28**	**9.31 ±0.60**	**9.24 ±0.42**	**9.17 ±0.13**	**8.88 ±0.13**
pH(50-60)	**9.85 ±0.16**	**8.92 ±0.25**	**9.59 ±0.27**	**9.72 ±0.19**	**9.20 ±0.15**	**9.38 ±0.22**
B(30-40)	**3.2 ±1.2**	0.39 ±0.09	**8.6 ±6.4**	**6.7 ±5.1**	**17.6 ±2.7**	0.43 ±0.17

Grain yield and screenings percentage

The mean site yields ranged from 360.3 g/plot (1.2 t/ha) at the AVH site to 839.3 g/plot (2.8 t/ha) at the RCL site, while the mean screenings percentage ranged from 4.0% at the RCL site to 9.97% at the AVH site (Table 3.2)

Table 3.2. Site mean yields (g/plot) and screenings (% below a 2.2 mm screen) for all plots included in the 2001 field trials.

Site	RHL	RHH	RCL	RCH	AVL	AVH
Yield	818 ±224	742 ±222	839 ±203	768 ±199	554 ±124	360 ±110
Screenings	5.6 ±2.9	7.3 ±5.1	5.0 ±2.5	8.0 ±4.6	3.6 ±1.9	9.8 ±5.3

The varietal correlations between sites for yield (Table 3.3) were all highly significant (P<0.001), but the AVH and AVL sites (shaded red) are clearly different from the other sites and correlate most closely (0.91) with each other. The other sites (shaded blue) correlate very closely with each other (0.96-0.99) and less closely with the Angas Valley sites (0.75-0.88).

Table 3.3 Varietal correlations between sites for grain yield, 2001. All correlations are significant (P<0.001)

	AVH	AVL	RCH	RCL	RHH
AVL	0.91				
RCH	0.77	0.88			
RCL	0.76	0.87	0.99		
RHH	0.76	0.86	0.96	0.98	
RHL	0.75	0.86	0.97	0.99	0.97

The varietal correlations between sites for screenings (Table 3.4) were lower than those for yield, but all were significant except for the relationship between the RHL and AVH sites.

Table 3.4. Varietal correlations between sites for screenings percentage, 2001.

	AVH	AVL	RCH	RCL	RHH
AVL	0.73***				
RCH	0.48***	0.69***			
RCL	0.32*	0.62***	0.97***		
RHH	0.71***	0.80***	0.91***	0.87***	
RHL	ns	0.36**	0.60***	0.72***	0.52***

* (P<0.05); ** (P<0.01); *** (P<0.001); ns not significant.

Relationships between soil characteristics and the grain yield and screenings percentage of Tamaroi check plots

Significant correlations were detected between soil characteristics and grain yield at all sites except the AVL site (Table 3.5), while significant correlations between soil characteristics and screenings percentage were only detected at three of the six sites (AVH, RCH and RCL) (Table 3.6). All of the correlations with screenings were positive, sugesting that salinity, boron and high pH all increased the percentage of screenings in the grain.

Table 3.5. Correlations (r) between soil covariates and the yield of thirty Tamaroi check plots at each of six experimental field sites in South Australia.

Covariate	Sites					
	AVH	AVL	RCH	RCL	RHH	RHL
EC(0-10)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
EC(30-40)	n.s.	n.s.	-0.768 ***	-0.485 *	n.s.	0.500 **
EC(50-60)	n.s.	n.s.	-0.848 ***	n.s.	0.40 *	0.517 **
pH(0-10)	-0.663 ***	n.s.	n.s.	n.s.	n.s.	-0.607 ***
pH(30-40)	n.s.	n.s.	-0.596 **	-0.401 *	-0.394 *	n.s.
pH(50-60)	n.s.	n.s.	n.s.	n.s.	-0.612 *	n.s.
boron	n.s.	n.s.	-0.698 ***	-0.58 ***	n.s.	0.544 **

ns not significant; * (P<0.05); ** (P<0.01); *** (P<0.001)

Table 3.6. Correlations (r) between soil covariates and the screenings percentage of thirty Tamaroi check plots at each of six experimental field sites in South Australia.

Covariate	Sites					
	AVH	AVL	RCH	RCL	RHH	RHL
EC(0-10)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
EC(30-40)	n.s.	n.s.	n.s.	0.569 ***	n.s.	n.s.
EC(50-60)	n.s.	n.s.	0.422 *	n.s.	n.s.	n.s.
pH(0-10)	0.586 ***	n.s.	n.s.	n.s.	n.s.	n.s.
pH(30-40)	n.s.	n.s.	n.s.	0.377 *	n.s.	n.s.
pH(50-60)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
boron	n.s.	n.s.	n.s.	0.519 **	n.s.	n.s.

ns not significant; * (P<0.05); ** (P<0.01); *** (P<0.001)

- Angas Valley, transient saline site (AVH)

The pH of the topsoil was significantly correlated with both the yield and percentage of screenings (-0.663 and 0.483 respectively) of the Tamaroi check plots (Tables 3.5 and 3.6) (Figures 3.3 and 3.4). This result was not unexpected as the mean pH in the 0-10cm sample was high (8.3) (Table 3.1) and ranged from 6.86 to 9.28, which included the pH value of 8.5, above which Peverill *et al.* (1999) claimed that the soils have a high concentration of free bicarbonate and poor nutritional status. The reduction in grain yield in response to increasing pH was also most pronounced above a pH of approximately 8.5 (Figure 3.3). The mean pH of the soils sampled from the 30-40 and 50-60cm depths were 9.81 ±0.11 and 9.85 ±0.16 respectively, indicating that root growth was almost certainly restricted to the soil above 30cm, possibly explaining the non-significant correlations with EC and B.

The mean boron concentration in the 30-40cm sample was only 3.2 ±1.2mg/kg and it was likely that it was much lower in the top soil and below the critical value of 3mg/kg (Peverill *et al.*, 1999). Similarly, the mean topsoil salinity of 0.22dS/m ($EC_{1:5}$) was unlikely to have any effect on plant growth and it was only in the deeper layers that salinity levels were over 0.5dS/m ($EC_{1:5}$).

Figure 3.3. The effect of topsoil pH (0-10cm) on the grain yield (g/plot) of Tamaroi check plots grown on a transient saline site at Angas Valley (AVH), 2003.

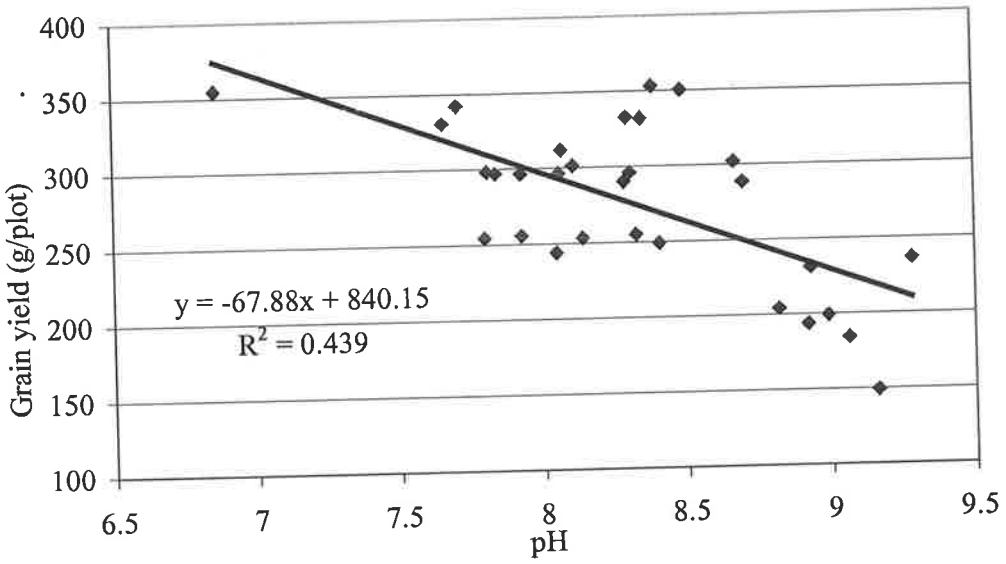
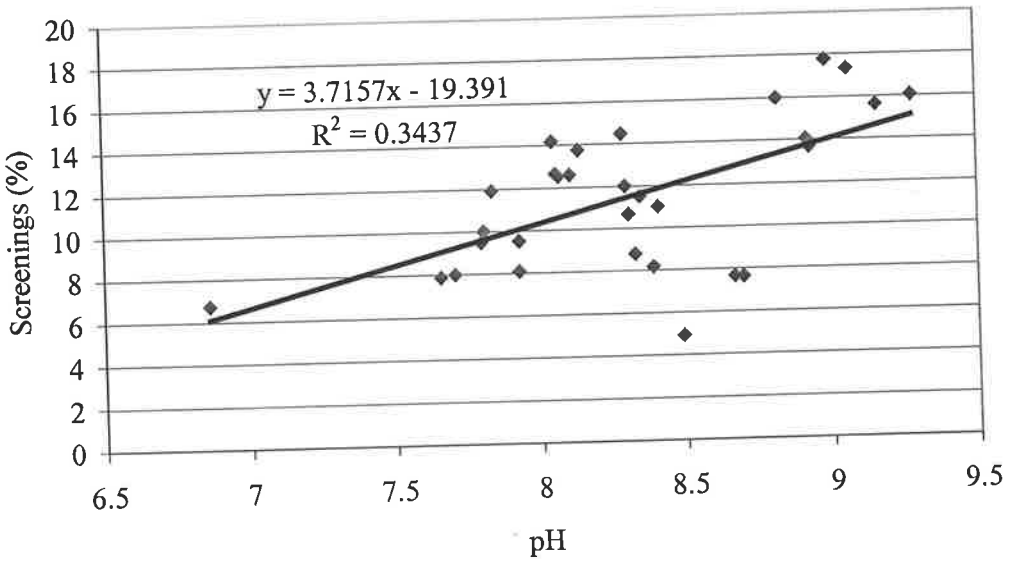


Figure 3.4. The effect of topsoil pH (0-10cm) on the percentage of screenings (<2.2mm) in grain of Tamaroi check plots grown on a transient saline site at Angas Valley (AVH), 2003.



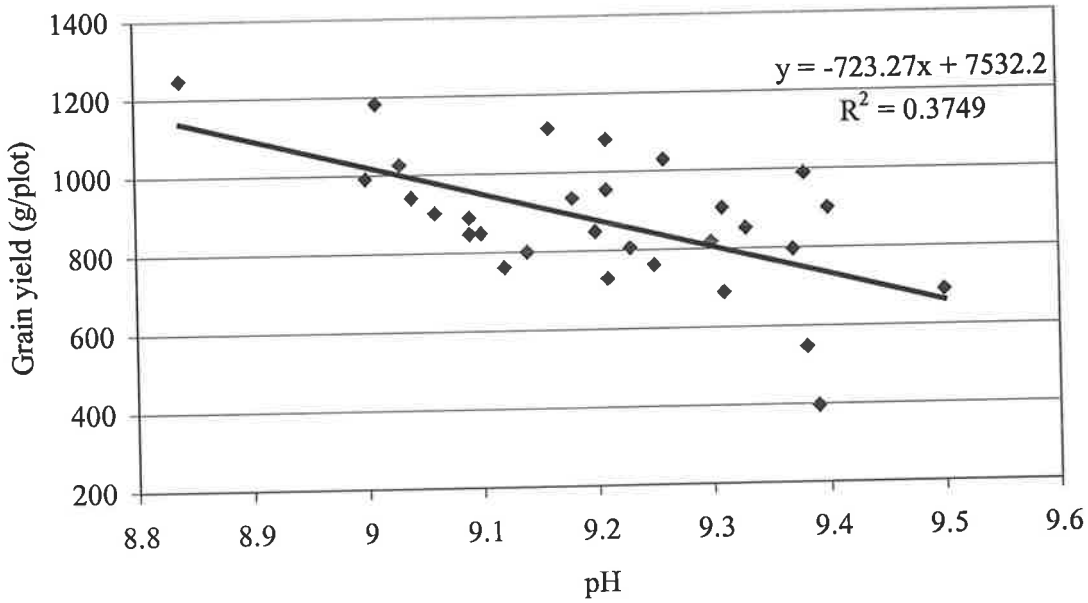
- Redhill, transient saline site (RHH)

Grain yield was negatively correlated with pH at both 30-40cm and 50-60cm ($P<0.05$) (Table 3.5; Figure 3.4). This was not unexpected, as the mean pH of the two depths was 9.17 ±0.13 and 9.2 ±0.15 respectively. The only other significant correlation was with salinity at the 50-60cm depth which, surprisingly, was positive, despite the mean $EC_{(1:5)}$ of 1.56 ±0.34 dS/m being the greatest of the six sites investigated.

There were no significant correlations between soil variate and screenings percentage (Table 3.6).

Multiple linear regression only retained pH(50-60) in the equation of best fit, which explained 35.6 % of the variation in yield. The other two covariates, pH(30-40) and EC(50-60) were correlated ($P<0.001$) with pH(50-60). The range in pH at a depth of 50-60cm (8.84-9.5) accounted for a difference in yield of 400g, or approximately 1.3t/ha (Figure 3.5).

Figure 3.5. The relationship between the pH of soil between 50 and 60cm and the grain yield of Tamaroi plots grown on a transient saline site at Redhill (RHH), 2003.



-Redhill, non-saline site (RHL)

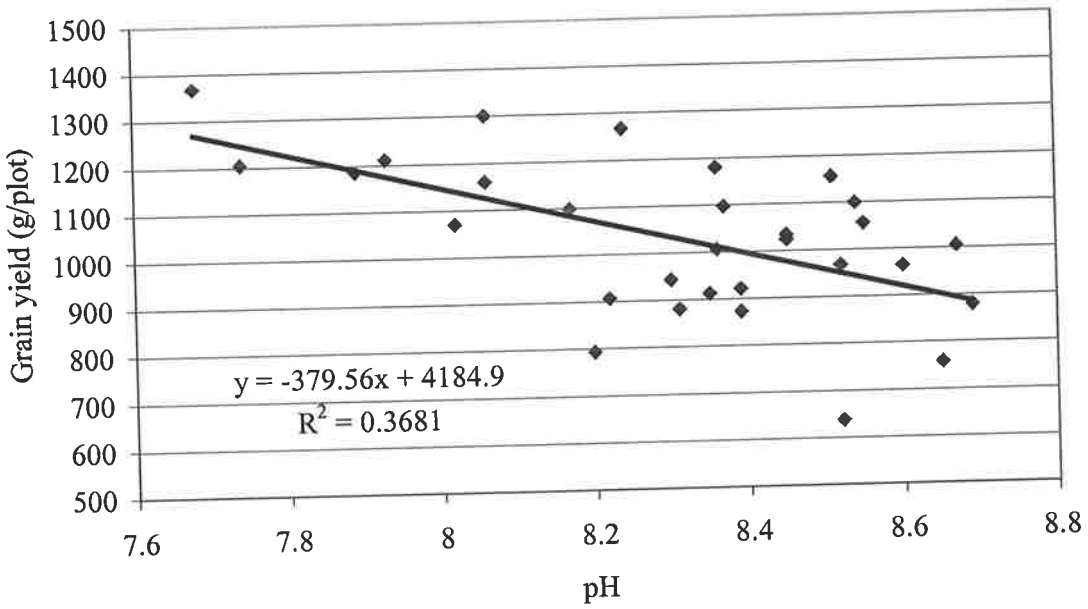
While the pH in the topsoil (0-10) was negatively correlated ($P<0.001$) with grain yield (Figure 3.6), three other covariates (EC(30-40), EC(50-60) and boron) were all positively correlated with grain yield ($P<0.01$) (Table 3.5). These three covariates all had mean values below the levels that were expected to have an impact on crop growth (Table 3.1). There were no significant correlations between soil covariates and screenings percentage.

Multiple linear regression was undertaken on the yield data to determine the equation of best fit, which explained 34.5% of the variation in grain yield. This equation only includes the covariate pH(0-10), which was negatively correlated ($P<0.01$) with the other three covariates that were significantly correlated with yield. The soil at this site consisted of a grey clay-loam of variable depth (approx. 20-100cm) over limestone rubble. Presumably, the depth of clay-loam was positively correlated with salinity, boron (both sub-critical) and

yield and negatively correlated with pH in the topsoil. It is likely that the plots with deeper A horizons had greater water availability to the plants.

The variation in pH(0-10) across the site ranged from 7.7 to 8.7, which resulted in a difference of 350g/plot, or approximately 1.2t/ha (Figure 3.6)

Figure 3.6. The relationship between the pH of soil sampled between 0 and 10cm and the grain yield of 'Tamaroi' plots grown on a non-saline site at Redhill (RHL), 2001.



- *Roseworthy, transient saline site (RCH)*

Several soil covariates were significantly negatively correlated with grain yield (Table 3.5). As it was suspected that many of these covariates were simply correlated with each other, and not necessarily contributing to the variation in yield, the data was subjected to multiple linear regression. The equation explaining the greatest percentage of variation in grain yield explained 70.9 percent of the variation and included only salinity at a depth of 50-60cm. All other covariates were excluded from the final equation, indicating that they do not explain any extra variation in yield. The correlations between the other covariates

(EC(30-40), pH(50-60) and B) and salinity at 50-60cm are all significant ($P<0.001$), supporting the hypothesis that the significant correlations with yield have primarily occurred through being associated with the covariate, EC(50-60) (Figure 3.7). As the pH(50-60) covariate appeared to have the dominant effect on yield at the RHH site (Figure 3.5), it was surprising that salinity had the closest relationship with yield at this Roseworthy site, considering that the pH at 30-40cm was higher than that measured at Redhill. The pH at both the 30-40 and 50-60cm depths was higher at the RCH site than the RHH site, while the $EC_{1:5}$ was lower, hence it could be expected that pH would have overridden the effects of salt. It is not possible to know why this discrepancy occurred, but it is clear that the relationship between EC(50-60) and grain yield (Figure3.7) is much closer than that of pH(30-40) and grain yield (Figure 3.8).

The significant effect of salinity at 50-60cm on grain yield was supported by the significant positive correlation of 0.422 ($P<0.05$) (Table 3.6) between this covariate and the percentage of screenings in the grain (Figure 3.9).

Figure 3.7. The relationship between salinity of soil sampled between 50 and 60cm and the grain yield of 'Tamaroi' plots grown on a transient saline site at Roseworthy (RCH), 2003.

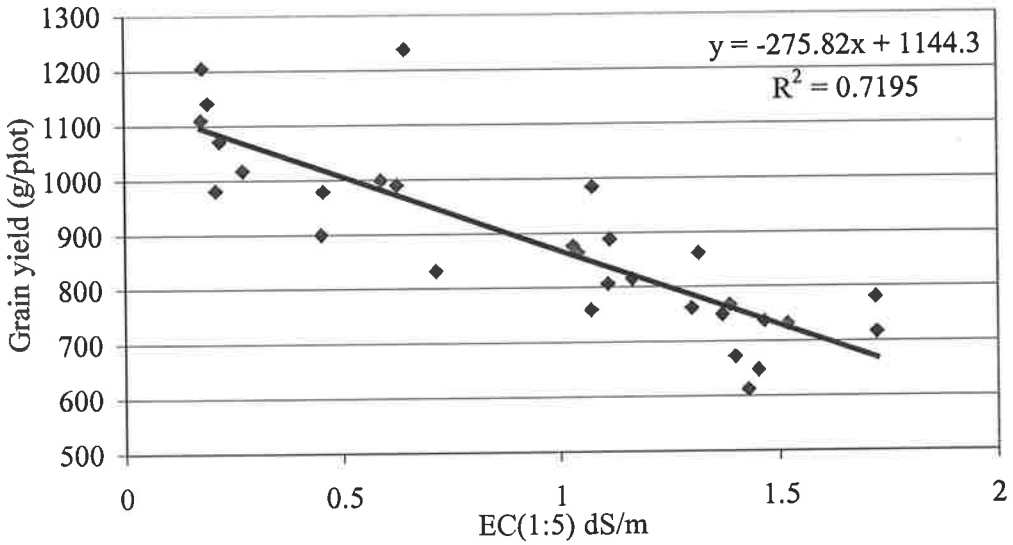


Figure 3.8. The relationship between the pH of soil sampled between 30 and 40cm and the grain yield of 'Tamaroi' plots grown on a saline site at Roseworthy (RCH), 2003.

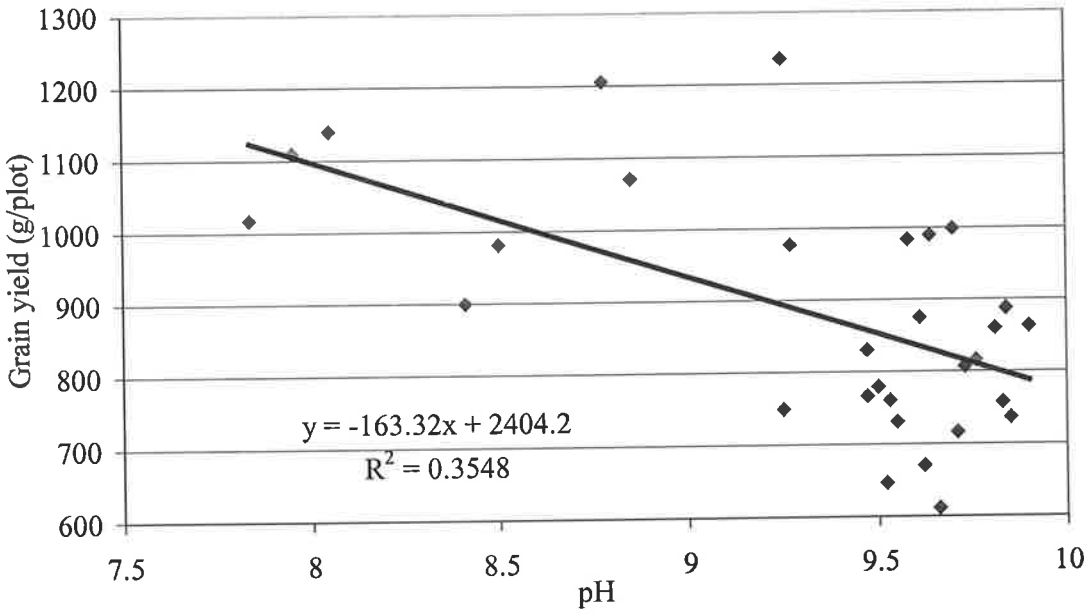
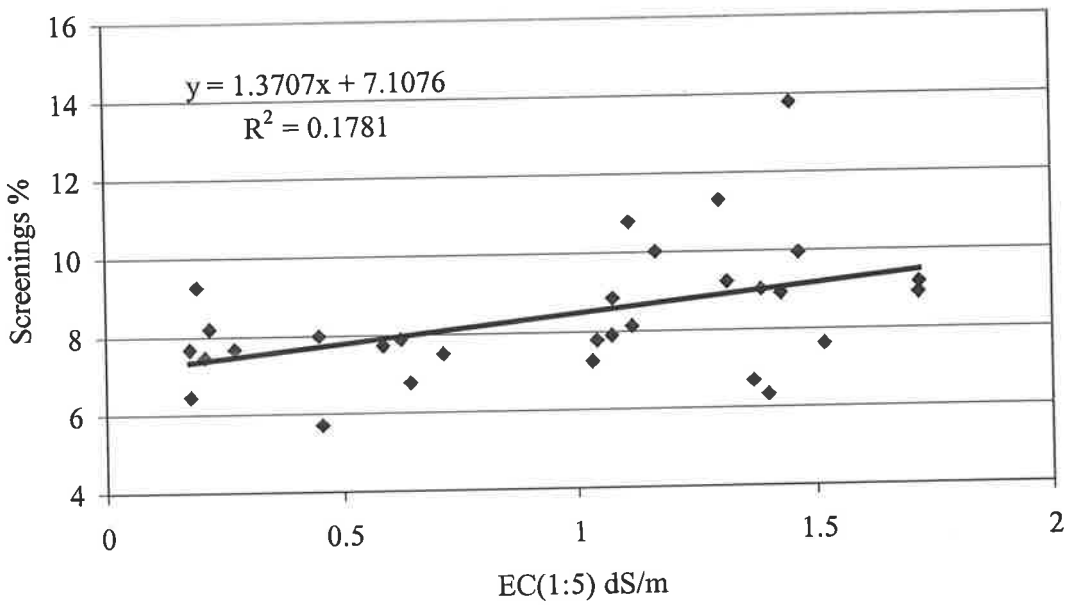


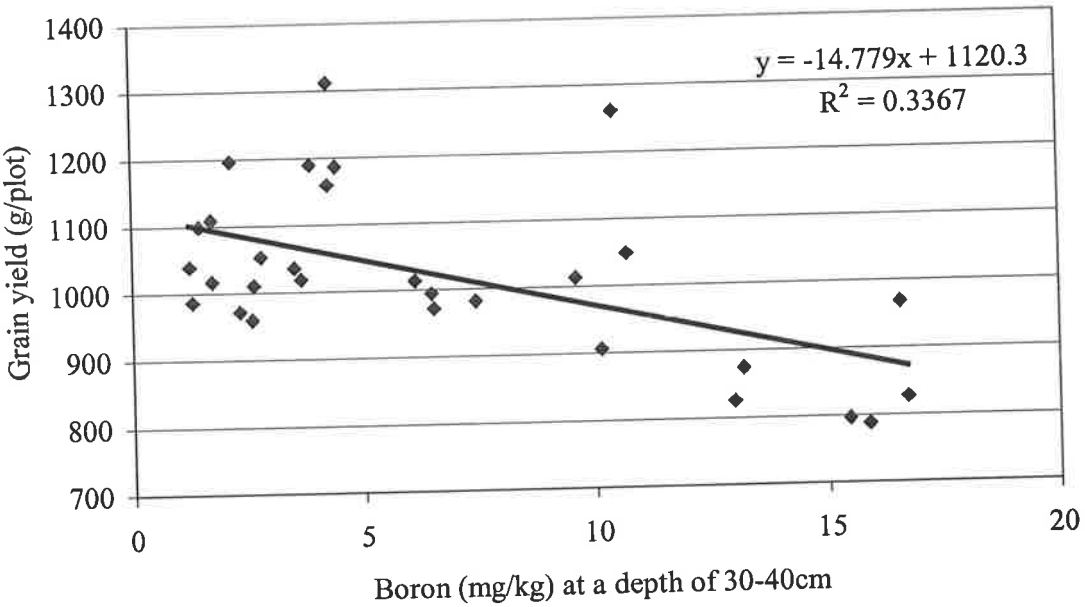
Figure 3.9. The relationship between salinity of soil sampled between 50 and 60cm and the screenings percentage (<2.2mm) in grain of 'Tamaroi' plots grown on a transient saline site at Roseworthy (RCH), 2001.



- Roseworthy, non-saline site (RCL)

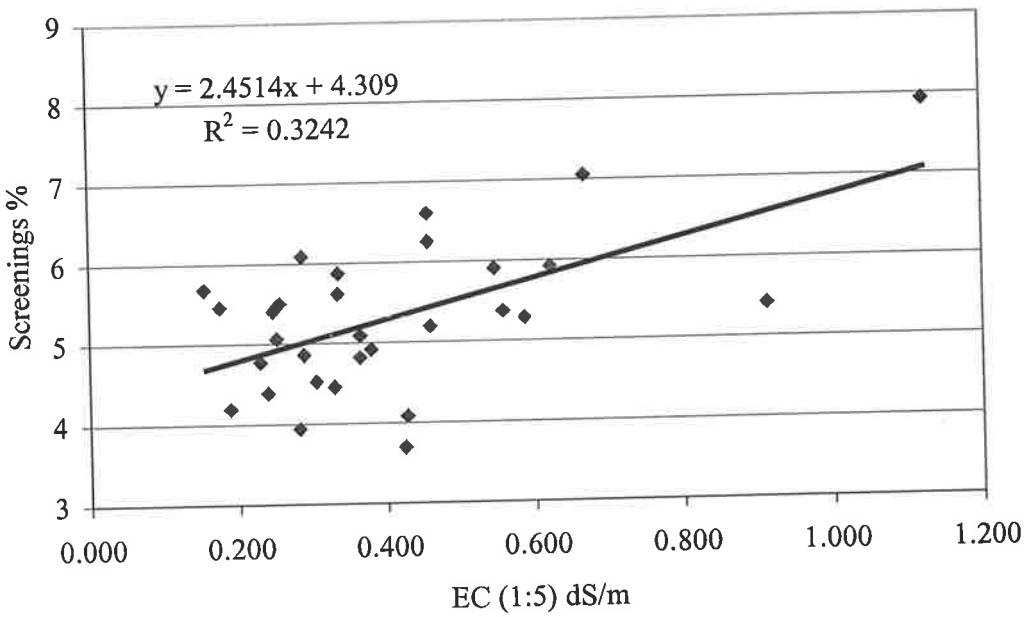
There were several soil covariates significantly correlated with the yield of the Tamaroi check plots at the RCL site, similar to the relationships observed at the RCH site (Table 3.5). Multiple linear regression was used again to identify the covariate/s explaining the greatest portion of the variation in yield. The final equation only retained the covariate boron, which explained 31.3% of the variation in yield (Figure 3.10). The other two covariates that were significantly correlated with grain yield (pH and EC at 30-40cm) were both significantly correlated with boron concentration ($P<0.001$). The relationship between boron concentration (at 30-40cm depth) and grain yield resulted in a yield reduction of approximately 200g/plot across the range of boron concentration measured (1.2-16.7mg/kg), which is approximately equivalent to 0.66t/ha (Figure 3.10).

Figure 3.10. The relationship between the boron concentration in soil sampled between 30 and 40cm and the grain yield of 'Tamaroi' plots grown on a non-saline site at Roseworthy (RCL), 2003.



The percentage of screenings in the grain was significantly correlated with the same three soil covariates as grain yield (Table 3.6). Multiple regression analysis was undertaken and in contrast to the regression undertaken for grain yield, the only covariate retained in the final equation was EC(30-40), which explained 30% of the variation in screenings. The other two covariates were correlated with EC(30-40) ($P<0.001$). The relationship between EC(30-40) and screenings resulted in an increase in screenings percentage of approximately 2% across the range of conductivity measured (Figure 3.11).

Figure 3.11. The relationship between salinity of soil sampled between 30 and 40cm and the screenings percentage (<2.2mm) in grain of 'Tamaroi' plots grown on a non-saline site at Roseworthy (RCL), 2003.



Genotypic differences

The relationships between both yield and screenings and the estimated soil covariates were plotted with the aim of identifying genotypic differences in tolerance from the slope of the line of best fit. While the slope varied between families, the differences were not significant, primarily due to the low number of observations. The Condor family, comprising the varieties Condor, Meering and Janz, tended to have a slope similar to that of the Tamaroi check plots. Conversely, the locally developed Aroona (Aroona, Schomburgk, BT-Schomburgk, Krichauff, Worrakatta and WI99072) and Spear (Spear, Frame, Yitpi, Trident, Stylet and Pugsley) families did not appear to suffer any reduction in yield.

3.2.4 Discussion

Despite the fact that these sites were chosen to represent areas affected by transient salinity, there was only one site where $EC_{1:5}$ was the statistically dominant covariate affecting yield (RCH) (Figure 3.7) and two sites where it had the dominant effect on the percentage of screenings (RCH and RCL) (Figures 3.9 and 3.11). This highlights the impact of the other subsoil constraints (high pH and boron toxicity) on field crop production in South Australia and the importance of combining tolerance to the three constraints in new durum and bread wheat varieties. An example of the importance of combining these tolerances is the fact that topsoil (0-10cm) pH was the best indicator of crop yield at the AVH site, despite the mean salinity and boron concentrations both being above their critical values at 30-40cm. It is highly unlikely that either boron or salt tolerance would have been of any value at this site, without the benefit of tolerance to high pH. Similar situations occurred at the RCH, RCL and RHH sites, where all constraints were present.

The impact of high pH on both grain yield and screenings was much greater than had been expected. Despite the predominance of high pH subsoils in this state, which as reflected in these field sites, the impact of high pH on plant growth in the field has been the subject of very little previous research. These results should increase the interest in the area in the future.

The low number of replicates resulted in non-significant relationships between the yield of individual bread wheat families and the covariates (those significantly affecting the Tamaroi check plots), even when the slope appeared to be large. In most cases, the grain

yield of the Condor family appeared to be affected by increases in pH to a greater extent than the Spear and Aroona families.

The Spear and Aroona families have dominated the wheat growing area of South Australia for more than a decade and have resulted from breeding work carried out in South Australia on soils which are now known to be affected by high boron, salt and pH. The Condor family (of interstate origin) has also been sown to large areas (mainly Janz); however, this has predominantly been restricted to the mid north and northern Mt. Lofty Ranges. These areas generally have less severe problems with sub-soil constraints such as salinity and high pH. Similarly, durum wheat is less well adapted to SA conditions than locally developed bread wheat varieties, mainly because of the short history of breeding and selection in this state.

Liu and Rathjen (1998) investigated the tolerance of a range of genotypes to bicarbonate ions in solution culture at a pH of 8.7 by measuring root growth. The results of their experiments indicated that the tolerance of Krichauff, BT-Schomburgk, W orrakatta (all Aroona derivatives) and Halberd (closely related to Spear) was much greater than that of the interstate varieties (Matong, Silverstar, and Olympic) and the durum varieties, Tamaroi and Yallaroi. Condor and its derivatives were not included in this work, but more recent experiments have shown them to be much less tolerant than the local varieties mentioned above (Rathjen and Das, unpublished). A population of random lines, derived by single seed descent from the cross between Yitpi (Spear family) and Meering (Condor selection), is now being produced and characterised for bicarbonate tolerance so that it can be used to measure the benefit of bicarbonate tolerance at high pH sites.

The lack of significant genotypic differences was primarily due to a lack of replication and possibly due to inaccurate estimation of the soil covariates for each treatment plot from the check plot data. If genotypic differences are to be investigated in the future, it is critical that replication is increased, as well as the intensity of soil sampling, possibly to the extent that every plot is sampled.

3.3 2002 Field experiments to investigate the effect of subsoil constraints on grain yield and screenings percentage.

3.3.1 Introduction

In 2002 a series of field experiments were undertaken that were similar to those of 2001. The 2002 field experiments aimed to obtain further information on the impact of the subsoil toxicities of transient salinity, high pH and boron toxicity at different sites, as well as defining genotypic differences in response. As it had been concluded that the lack of significant genotypic effects in the 2001 experiments resulted from the soil covariates only being measured on the Tamaroi check plots and that there was insufficient replication, the 2002 experiments aimed to overcome these problems by sampling the soil more intensively and by including more replicates of each genotype.

3.3.2 Materials and methods

The field experiments were sown at Redhill (RH), Jamestown (JC) and a high and low salt site at Roseworthy (RCH and RCL). Ten replicates of fifteen bread wheat and three durum genotypes were included in each experiment. Tamaroi check plots were included every fifth plot, from plot two of the odd numbered bays and plot four of the even bays. The experiments were deliberately located on sites which had variable levels of salinity, so that

the response of individual genotypes to variation in the covariates (particularly salt) could be estimated by regression analysis. The total number of check plots was forty-five, hence the total number of plots was 225, arranged into an array of fifteen bays and fifteen columns. The experiments were sown using the machinery and methods of the Waite wheat breeding program.

After harvest, the grain from each plot was weighed and cleaned using a Carter-Day® Dockage tester and sieved over a 2.2mm screen. The grain passing through the screen was weighed and the percentage of screenings calculated.

Soil samples were taken from the 0-10cm and 35-55cm depths of a single core from each plot in the odd numbered columns (1,3,5,....,15). Electrical conductivity and pH were measured on 1:5 soil paste extracts from all samples, while extractable boron in hot $CaCl_2$ (Spouncer *et. al.,* 1992) was measured on the 35-55cm soil samples only. The EC(1:5), pH and boron concentration of the plots in even numbered columns was estimated from the mean of the neighbouring two plots. It was expected that this estimate would be reasonably accurate because each plot was only four rows (60cm) wide, hence the sampling interval was 120cm.

Statistical analyses were performed using the same methods described in Section 3.2.2 of this chapter.

3.3.3 Results

Soil characteristics

The soil covariate mean values have been tabulated in Table 3.7, with the values that were expected to restrict plant growth highlighted in bold, as described in Section 3.2.3.

Table 3.7. Site mean $EC_{1:5}$ (dS/m) (measured at 0-10 and 35-55cm), pH (0-10 and 35-55cm) and boron (mg/kg) (35-55cm) values. Soil salinity >0.5dS/m $EC_{1:5}$ (Maas and Hoffman, 1977), pH >8.5 (Peverill *et al.* 1999) and boron >3mg/kg (Peverill *et al.* 1997) that were expected to restrict crop growth have been highlighted.

Covariate	Sites			
	JC	RH	RCL	RCH
EC(0-10)	0.09 ±0.02	0.27 ±0.12	0.13 ±0.01	0.126 ±0.04
EC(35-55)	0.31 ±0.13	**1.21 ±0.33**	0.16 ±0.02	**1.20 ±0.33**
pH(0-10)	6.8 ±0.3	6.5 ±0.2	7.2 ±0.5	6.9 ±0.3
pH(35-55)	7.9 ±0.3	**9.1 ±0.2**	**8.7 ±0.1**	**9.1 ±0.2**
Boron	0.17 ±0.14	**7.4 ±1.5**	0.2 ±0.1	2.2 ±1.4

Grain yield and screenings percentage

The 2002 crop was severely affected by drought across South Australia, which resulted in below average yields. The grain yields at the four field sites included in these experiments ranged from 269 ±86g/plot at the saline Roseworthy site to 366 ±124g/plot at Jamestown, which was approximately equivalent to 0.9 and 1.2t/ha respectively (Table 3.8).

Table 3.8. Mean grain yield (g/plot) and the percentage of grain passing through a 2.2mm screen of all plots in the 2002 experiments.

Site	Grain yield (g/plot)	Screenings (%)
Redhill	330 ±137	21.3 ±8.5
Jamestown	366 ±124	18.7 ±6.5
Roseworthy (saline)	269 ±86	25.8 ±8.3
Roseworthy (non-saline)	310 ±73	22.4 ±5.5

Relationships between soil characteristics and the grain yield and screenings percentage of Tamaroi check plots

Only at the Jamestown site was there any significant correlations detected between soil covariates and either the grain yield or screenings of the Tamaroi check plots (Table 3.9). At this site the EC(0-10) was negatively correlated with yield ($P<0.001$) and positively correlated with screenings ($P<0.001$), while the pH(35-55) was positively correlated with yield ($P<0.05$) and negatively correlated with screenings ($P<0.01$).

Table 3.9. Correlations (r^2) between soil covariates and the yield and screenings percentage of forty-five Tamaroi check plots at Jamestown, 2002.

Covariate	Yield	Screenings %
EC(0-10)	-0.50 ***	0.54 ***
EC(35-55)	ns*	ns
pH(0-10)	ns	ns
pH(35-55)	0.35 *	-0.44 **
boron	ns	ns

* not significant; * (P<0.05); *** (P<0.001)

Multiple linear regression on the grain yields of Tamaroi check plots at Jamestown explained 23.5% of the variation and only included the EC(0-10) covariate (Figure 3.12). This relationship between EC(0-10) and yield resulted in a difference of approximately 250g/plot (0.8 t/ha) across the range in $EC_{(1:5)}$ measured (Figure 3.12).

Multiple Linear regression analysis was also undertaken on the screenings data from Tamaroi check plots at Jamestown. This identified an equation of best fit which explained 37.1% of the variation and retained both the EC(0-10) and pH(35-55) covariates (Table 3.10). The individual relationships between EC(0-10) and screenings (Figure 3.13) and pH(35-55) and screenings (Figure 3.14) each resulted in a difference of approximately 15% in screenings across the ranges measured.

Figure 3.12. The relationship between salinity of the soil sampled between 0 and 10cm and the grain yield of forty-five 'Tamaroi' plots grown at Jamestown, 2002.

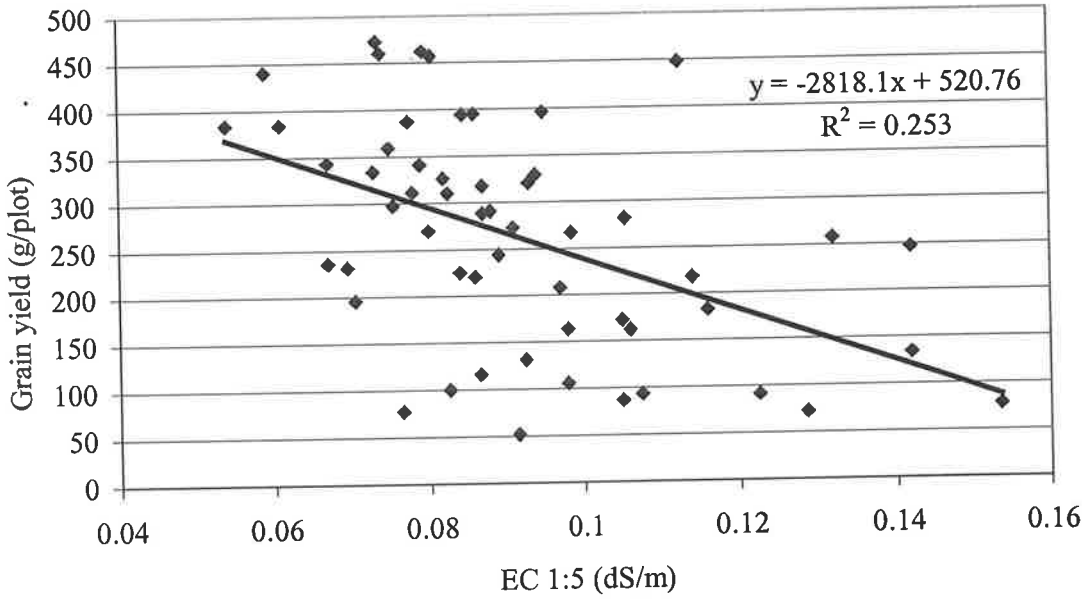


Table 3.10. Parameter estimates of the equation best describing variation in screenings percentage (<2.2mm) in the grain of forty-five Tamaroi plots grown at Jamestown, 2002.

	Estimate	s.e.	t(28)	t pr.
Constant	66.7	18.7	3.56	<0.001
EC(0-10)	0.1356	0.0329	4.12	<0.001
pH(35-55)	-6.75	2.24	-3.02	0.004

Figure 3.13. The relationship between salinity of the soil sampled between 0 and 10cm and the screenings percentage (<2.2mm) of grain of forty-five 'Tamaroi' plots grown at Jamestown, 2003.

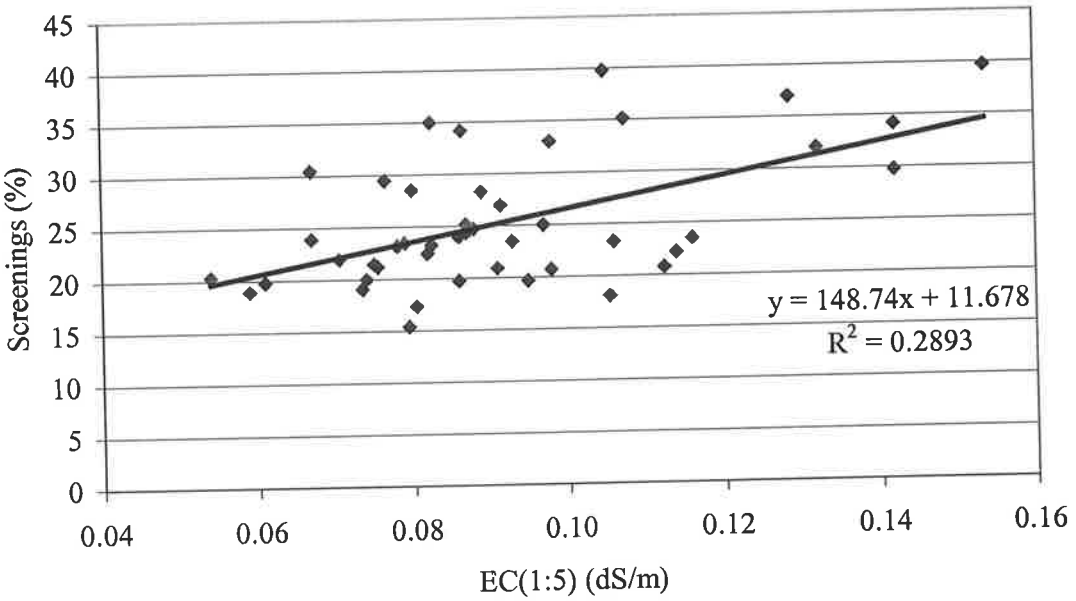
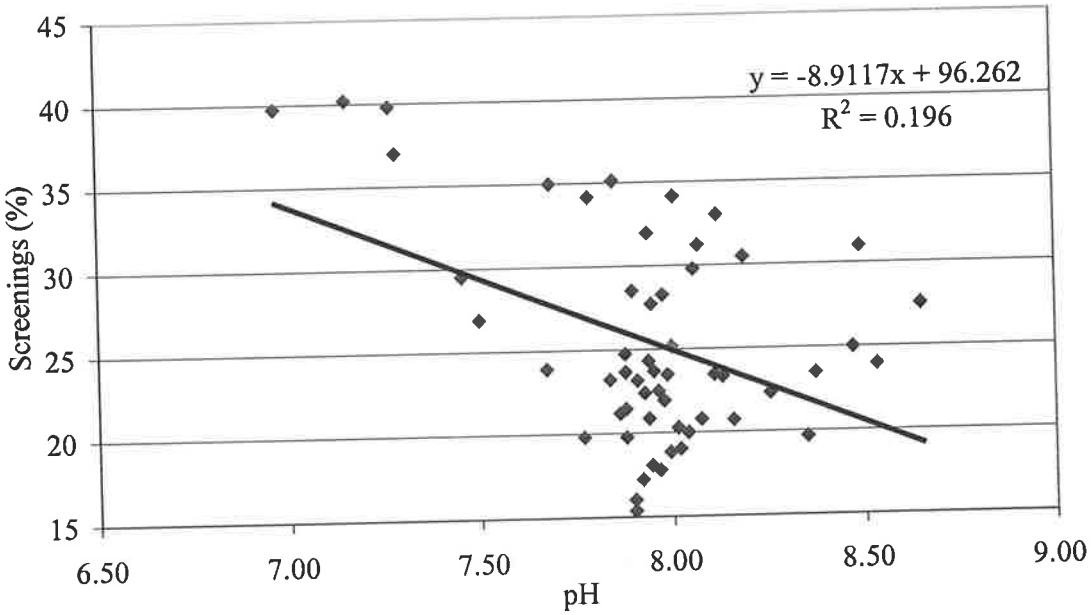


Figure 3.14. The relationship between pH of the soil sampled between 35 and 55cm and the screenings percentage (<2.2mm) of grain of forty-five 'Tamaroi' plots grown at Jamestown, 2002.



Genotypic differences

The yield and screenings percentage of different genotypes were plotted against EC(0-10), but no significant relationships were detected.

3.3.4 Discussion

The number of soil covariates that were significantly correlated with the grain yield or screenings percentage of the Tamaroi check plots was much lower than the previous year. In the very dry conditions it is likely that little water recharged the subsoil. Consequently, the plants would not have utilised subsoil moisture during grain fill and the three subsoil constraints would have been largely irrelevant to plant growth.

Significant correlations were detected between EC(0-10) and both yield and screenings at Jamestown, despite the mean $EC_{1:5}$ of 0.09 ±0.02 dS/m being well below the critical value of Maas and Hoffman (1977). It is possible that under the dry conditions experienced in 2002, that the salt concentration in the soil water was actually much higher than was indicated by the $EC_{1:5}$ measurement (Rengasamy *et al.*, 2003).Alternatively, it is possible that the variation in yield was actually correlated with some other soil character that was correlated with the topsoil salinity.

Variation in soil structure, which may have an effect on crop growth, was observed across the site. The subsoils varied from a moderately friable brown clay-loam to a sodic red clay loam, which had very poor structure and apparently did not contain plant roots. The red clay subsoil was not mapped, but it appeared to be co-incident with the areas of higher surface salinity. The section of the paddock on which the experiment was located had been affected by high water table levels in 1981 and 1992, which caused the surface soils to

become so saline that large areas were scalded (bare) for many years. After the water table fell, rainfall flushed the salt back down the profile over successive years, until 2000 when the whole paddock was successfully cropped. The poorly structured red clay subsoils would have allowed less movement of water down through the profile, resulting in less leaching and more retention of salt in the topsoil. Following the observation that the red clay subsoil restricted rooting proliferation and depth and thereby plant available water, it was not surprising that topsoil salinity was positively correlated with yield and negatively correlated with screenings.

The range of pH measured in the 35-55cm samples (6.9-8.6) from Jamestown was unlikely to have any negative effect on crop growth. The correlations with yield and screenings (Table 3.9) were more likely to be due to an association with the variation in soil structure described above, or with another unidentified character.

3.4 Discussion of Chapter 3.

The results of these field experiments have shown that the subsoil constrains transient salinity, boron and high pH, did have a significant negative impact on durum production. This impact was probably dependant on seasonal conditions, such as the amount of subsoil moisture present during grain fill. For example, in 2002 only limited water would have actually recharged the subsoil due to the drier than average growing season. Under these circumstances the presence or absence of subsoil constraints was likely to have been largely irrelevant. Conversely, during a grainfill period of higher than average rainfall, the crop may be able to extract all of the water required from the topsoil, without any need to access subsoil moisture.

The dominant subsoil constraint affecting grain yield or screenings had a level above the published critical value at all field sites investigated in 2001 (Tables 3.5 and 2.6). However, the presence of transient salinity, boron, or pH above their critical values, did not ensure that any individual constraint was the dominant yield limiting factor. In many cases, more than one of the three constraints was present (Table 3.1). This may explain situations where boron tolerant genotypes have not had an advantage over intolerant genotypes when grown in boron toxic soil (Jefferies, 2000; Wheeler pers comm.).

The saline Angas Valley site provided a very good example of the importance of measuring all three subsoil constraints, as all of these could have been expected to limit yield. At this site, the topsoil pH was greater than 8.5, over a large portion of the experimental area and this was the covariate which had the closest relationships with grain yield and screenings. At the depth where salinity and the concentration of boron reached yield limiting levels, the pH was in excess of 9.8 and undoubtedly the latter would have completely restricted root growth. Under these conditions, breeding for either salt or boron tolerance is unlikely to be of any benefit in the absence of tolerance to high pH.

The sites where high pH was shown to be the factor limiting crop productivity were more common than expected, especially at the sites selected specifically for transient salinity. Further work is needed to identify the actual effects of high pH on plant growth, but recent work by Rathjen and Das (unpublished) has identified durum landraces that are more tolerant to bicarbonate ions. This trait, and any others suspected of conferring tolerance to high pH, will need to be backcrossed into backgrounds containing tolerance to both transient salinity and boron tolerance before it is possible to accurately assess the benefit of high pH tolerance in the field.

Breeding durum varieties with a combination of tolerance to these three subsoil constraints is of paramount importance if the adaptive range of the crop is to be expanded beyond the areas where it is currently grown. It is also possible that other chemical and physical constraints not considered in this study, also affect the rooting depth and the availability of subsoil moisture in these soils. These will need to be elucidated and investigated. The release of the boron tolerant variety 'Kalka" in 2003 is a step towards better adaptation of the durum crop, but it is unlikely that this variety will have any substantial benefit over previous varieties on soils affected by transient salinity or high pH.

Chapter 4.

THE GENETIC CONTROL OF Na EXCLUSION IN THE BREAD WHEAT VARIETY 'KRICHAUFF' AND ITS AGRONOMIC IMPORTANCE.

4.1 Introduction

Two basic mechanisms for salt tolerance in plants have been documented; reduced transport of salt to the shoots and tolerance to high leaf salt concentrations by sequestration within cell vacuoles (Greenway and Munns, 1980).

Na accumulates in the leaves of cereals as a result of transpirational flow from the roots. As salt from transpirational flow accumulates over time, young leaves tend to have lower ion concentrations than older leaves. If the plant accumulates high cytoplasmic levels of Na ions, inhibition of metabolic enzymes occurs and eventually the older leaves become necrotic (Greenway and Munns, 1980). Clearly genotypes that are able to exclude Na from the roots are likely to be more tolerant of saline growing conditions than other genotypes.

The agronomically important sister varieties Krichauff and Worrakatta are highly adapted to all areas of the state and have been particularly important to farmers on the Eyre Peninsula and in the Murray Mallee. This is at least partly due to their improved tolerance to the subsoil constraints boron toxicity (Primary Industries and Resources SA, 1999) and sodium bicarbonate (Liu and Rathjen 1998; Rathjen and Das unpublished data). The tolerance of these varieties to transient salinity has not been investigated.

These two varieties are very closely related sister lines, reselected in the F_5 generation from the same F_2 derivative. One difference often observed between the two varieties, is that Worrakatta has a tendency to produce smaller, shriveled grain, more frequently than Krichauff. At many, but not all sites Worrakatta has higher levels of screenings than Krichauff, resulting in Worrakatta grain having 40% more screenings when averaged over all sites in the South Australian stage four trials (South Australian research and Development Institute, 1997, 1998, 1999, 2000, 2001) (Table 4.1).

Table 4.1. Screenings percentage of grain harvested from SARDI stage four trials. Percentage of grain passing through a 2.2mm screen (1996-7) and 2.0mm screen (1998-2000) (total of 100 observations).

	Year					
	1996	1997	1998	1999	2000	Mean
Krichauff	7.55	8.61	1.43	2.4	2.1	4.42
Worrakatta	9.54	12.72	2.43	3.5	3.1	6.26
Worrkatta Screenings as a % of Krichauff	126	148	170	146	148	142

(South Australian Research and Development Institute, 1997, 1998, 1999, 2000, 2001)

As part of an investigation into genetic variation for Na accumulation, whole tillers of a range of durum and bread wheat varieties were sampled from field experiments located at Angas Valley and Redhill and analysed by ICP-spectrometry. The data obtained suggested higher accumulation of Na in Worrakatta than Krichauff. Analysis of variance on this data indicated significant genotypic differences, but this was due to the inclusion of the durum lines in the

data, which typically have a Na concentration in the whole shoot five to ten times higher than that of bread wheat. When the bread wheat genotypes were analysed independently, statistically significant genotypic differences were not detected, but the ranking of varieties for Na concentration reflected earlier results.

Na accumulation data for whole tillers of thirty-nine lines had also been collected from a field trial at Two Wells by Chao-Yin Liu in 1997 (unpublished). In this trial, Worrakatta had accumulated almost twice as much Na as Krichauff (935 compared to 501 mg/kg), ranking them at opposite ends of the range of bread wheat genotypes tested. As samples from three replicates had been bulked into a single sample prior to testing, statistical analysis could not be undertaken.

On the basis of the possible difference in Na accumulation between Worrakatta and Krichauff a series of experiments were undertaken to determine:

(1) Whether a statistically significant difference in the Na accumulation between the sister varieties Worrakatta and Krichauff could be confirmed.

(2) The genetic basis of the difference in Na accumulation between the two varieties.

(3) Whether the differences in screenings % that is observed at some sites was the result of differences in Na accumulation.

(4) If the reduction in Na accumulation from Worrakatta to the Krichauff level would have a significant effect on grain yield under transient saline conditions.

4.2 Comparison of Na accumulation in the hexaploid wheat varieties 'Worrakatta' and 'Krichauff'.

4.2.1 Introduction

A pot experiment was conducted in the glasshouse to determine whether a significant difference in Na accumulation existed between the sister varies Worrakatta and Krichauff. The advanced breeding line WI99072, developed from a cross between a Krichauff derivative and Worrakatta, was included in the experiment to assess its Na accumulation relative to the two parents.

4.2.2 Materials and methods

Seed

The seed used was obtained from the SARDI Field Crop Evaluation Unit and was pre-germinated at 4°C for three days, followed by 20°C for twenty-four hours.

Soil

Three kilograms of air dried University of California mix from SARDI Plant Growth Services was weighed into each of thirty-six 175mm diameter pots lined with plastic bags.

Experimental Design

The pots of the three genotypes and two salt treatments were combined factorially and arranged in six randomized complete blocks on a glasshouse bench. Six pre-germinated seeds were planted in each pot, which were thinned to five after eight days to enhance uniformity. At eight days, 150mL of 0.5M NaCl solution was added to each pot receiving the saline treatment, while 150mL of RO water was added to the non-saline pots. Consequently, the saline pots had an electrical conductivity of approximately 0.9 dS/m ($EC_{1:5}$), or 9 dS/m (ECe), while the conductivity of the control pots was less than 0.1dS/m ($EC_{1:5}$). The experiment was limited to two levels of salinity, allowing more replication without the cost of analysis using ICP-spectrometry being restrictive on future experiments.

To avoid waterlogging (the plastic pot liner did not allow drainage) and to maximise Na uptake, watering was performed only when the plants began wilting. Each pot was watered with 150mL of RO water. Clear plastic sheeting was suspended from the roof of the glasshouse to deflect water leaking through the roof during rainfall and thereby avoid rainwater affecting the concentration of NaCl in the soil solution of individual pots.

Plants were harvested at anthesis, washed in RO water, oven dried at 70°C, ground in a stainless steel mill and sieved through a 2mm screen for analysis using ICP-spectrometry by Waite Analytical Services.

4.2.3 Results and Discussion

Analysis of variance revealed that the effect of genotype, salt treatment and the genotype x salinity interaction all had a significant effect ($P<0.01$) on whole plant Na concentration.

Based on the LSD (5% level), the three genotypes were not significantly different from each other under the low salt treatment, while under the high salt treatment, all genotypes had significantly higher Na concentration than the control and were significantly different from each other (Table 4.2). In the high salt treatment, WI99072 had significantly higher Na accumulation than Krichauff, and Worrakatta had significantly higher Na concentration than both of the other genotypes.

Table 4.2. Na concentration (mg/kg) of whole plants at anthesis, of three genotypes grown in pots in a glasshouse at two levels of salinity.

Genotype	$EC_{1:5}$	
	0.1	0.9
Krichauff	345	928
WI99072	317	1305
Worrakatta	403	1857
LSD (5% level) 366.8		

These results confirm the indications from field data, that there is a significant difference in Na accumulation between Worrakatta and Krichauff. This justified the development of a population from the cross between the two varieties to study the inheritance of the Na exclusion of Krichauff and to investigate if this were responsible for the reduced grain shriveling observed in Krichauff at some sites.

4.3 Development of a population from the cross (Worrakatta*Krichauff).

4.3.1 Introduction

Reduced sodium accumulation could be expected to have some effect on field response to salt toxicity (Greenway and Munns 1980) and as the previous experiment had shown that Worrakatta accumulated significantly more Na in the whole shoot than its sister variety Krichauff, this hypothesis could be tested.

A population was developed from the cross (Worrakatta*Krichauff) to determine the number of genes controlling the Na exclusion trait and whether this level of sodium exclusion resulted in a significant improvement in field tolerance to transient salinity. If a significant difference in salt tolerance were associated with reduced sodium accumulation, we must consider whether introducing additional sodium exclusion genes into the Krichauff background would provide additional tolerance to transient salinity. An obvious candidate gene for such a project is the Na exclusion gene identified in the durum landrace Na49 (see Chapter 5).

4.3.2 Materials and methods

The cross (Worrakatta*Krichauff) was made by the Waite wheat breeding program, under the leadership of Dr. A.J. Rathjen in 1995. A single F_2 bulk plot was sown at Roseworthy in 1996, from which sixty heads were selected at random. These heads were sown directly as head hills in the Waite bird proof enclosure over the 1996/7 summer (F_2 derived F_3). In May 1997, the sixty F_2 derived selections were sown in a single replicate experiment on the property of Mr. C Hazel of Kapunda in 4 row x 4.2m plots.

In December 1997 these plots were harvested, providing F_2 derived F_5 seed which was stored at 12°C in the Waite Wheat Group seed store until November 2001. Five seeds were selected at random from each F_2 derived family and planted in 250mm pots. These were thinned to four plants after emergence. F_2 selections 9, 31 and 32 failed to germinate and consequently were not reselected, so the total number of $F_{2\text{-}5}$ selections was 228. The F_5 derived F_6 seed from the single plants was multiplied in the Waite Campus polyhouse. The F_5 derived lines were sown in a non-replicated field experiment on the twentieth of August at Roseworthy, primarily to provide seed for further field experiments. Spring rainfall was well below average and this resulted in a mean grain yield of only 276 ±73g/plot or 0.93 t/ha. The low yield restricted the 2003 field experiments to 141 lines, grown at four sites.

The initial (Worrakatta*Krichauff) cross was made more than six years prior to the development of the population and long before the difference in sodium acccumulation of the two parents had been identified. To check that the parentage of the cross was between a high Na accumulating Worrakatta plant and a Na excluding Krichauff plant, a pot experiment was conducted to ensure that the F_2 families were segregating for sodium exclusion.

The experiment described in Section 4.2 determined that the difference in Na accumulation between the two parents could be detected at approximately 0.9 $dS/EC_{1:5}$. On this basis only one salt treatment was used in this experiment to reduce the total cost of ICP-spectrometry analysis, while maintaining adequate replication.

Pots and soil

As described in Section 4.2 of this chapter.

Seed

Fifteen F_5 seeds from each of the F_2 derived families were pre-germinated on moist filter paper in Petri dishes, at 4°C for three days, followed by 20°C for twenty-four hours.

Experimental design

The pots were arranged in a glasshouse in three completely randomised blocks of the fifty-seven F_2 families, plus one pot of Krichauff and two pots of Worrakatta. Seven pre-germinated seeds were planted in each pot, which were thinned to six at day eight, when the addition of salt occured. Six plants were retained in each pot because it was expected that a high percentage of the families would be heterogeneous.

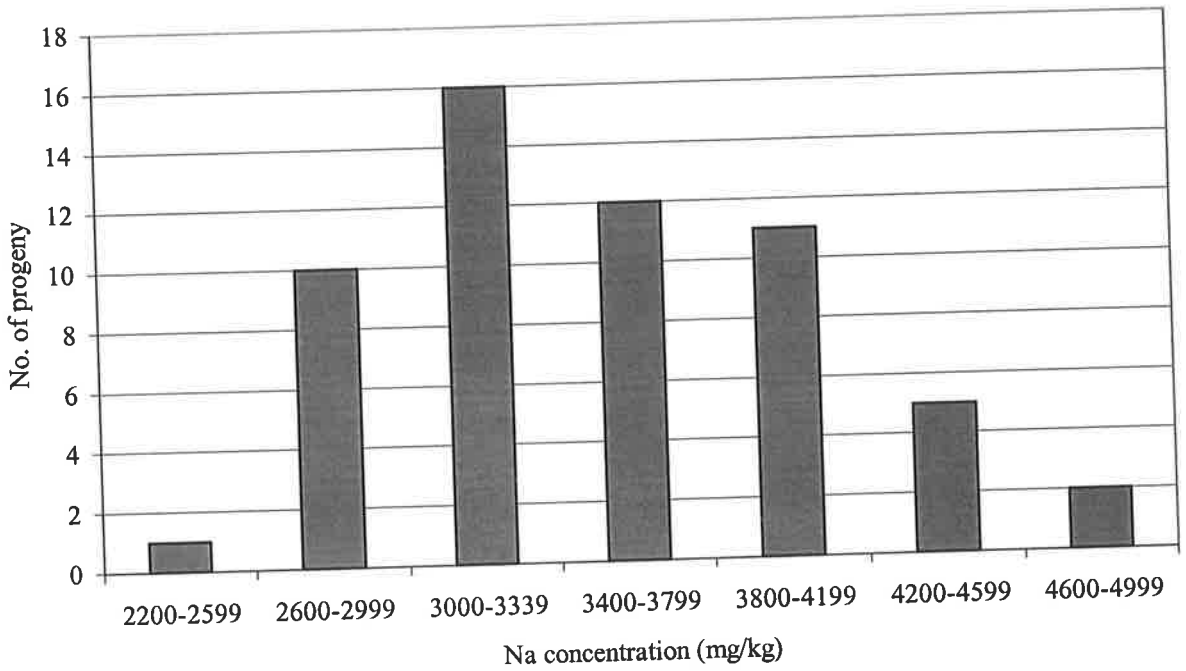
Salt addition, watering, harvesting of plant material and ICP-spectrometry analysis were performed in the same way as the experiment described in Section 4.2 on plants harvested at anthesis. Worrakatta and Krichauff are very closely related sister varieties; consequently the progeny from the (Worrakatta*Krichauff) cross were not segregating for maturity, allowing all lines to be harvested at the same time.

4.3.3 Results and discussion

Analysis of variance of whole plant Na concentration indicated that genotype had a significant effect ($P<0.01$). The mean Na concentrations of Krichauff and Worrakatta were 2633 ±586

and 4767 ± 748 mg/kg respectively, while the mean of the F_2 derived progeny was 3459 ±933 mg/kg. The LSD ($P<0.05$) of 1488 mg/kg indicates that the mean Na concentrations in the whole tillers of Worrakatta and Krichauff were significantly different from each other. The distribution of F_2 derived F_5 lines for Na concentration was inclusive of the parental means, but there was no indication of segregation into distinct classes (Figure 4.1).

Figure 4.1. Distribution of F_2 dervied F_5 progeny from the cross (Worrakatta*Krichauff) for Na concentration in the whole shoots of plants at anthesis. Plants grown in pots with an electrical conductivity of 9dS/m (ECe) in a glasshouse.



This result confirmed that the cross had been made between plants that were genetically different for Na exclusion, by virtue of the fact that the range of Na concentration observed in the F_2 derived progeny was inclusive of the significantly different parental means. Consequently, the 228 $F_{2,5}$ derived lines were also segregating for the Na exclusion trait.

4.4 Investigation into the effect of soil salinity on the grain size and yield of Worrakatta and Krichauff.

4.4.1 Introduction

It had been shown previously that Worrakatta accumulated significantly more Na in the whole shoot than its sister variety, Krichauff, when grown in saline soil (Section 4.2). A pot experiment was conducted in the glasshouse to test the hypothesis that the higher Na accumulation of Worrakatta resulted in reduced grain yield and size relative to Krichauff under saline conditions.

4.4.2 Materials and methods

Pots and soil

Air-dried, pasteurized sand from Palmer was mixed with water in an electric concrete mixer to achieve an even 10% moisture content, before 7.5 kg was weighed into each of thirty-two 250mm pots. The pots had been lined with plastic bags to prevent leaching of salt from the soil. Palmer sand was chosen as the growth media because it has high water holding capacity, and is not subject to non-wetting problems or toxicities such as high pH or boron (Rathjen pers. comm.).

Experimental procedure

The experiment included the varieties Worrakatta and Krichauff and four salt treatments (0, 4, 8 and 12g of NaCl/pot), which were combined factorially and replicated three times in randomized complete blocks. One hundred seeds of each variety were pre-germinated on

moist filter paper in Petri dishes for six days prior to planting. Four seedlings were planted in each plot.

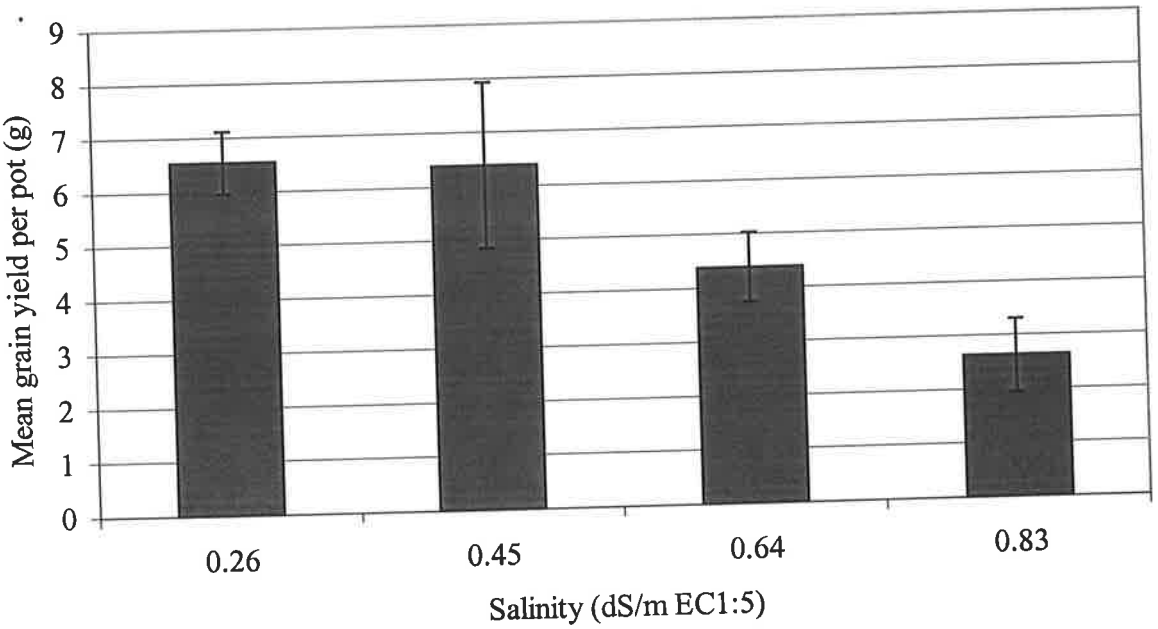
The pots were re-watered to the starting weight of 7750g (7.5kg soil plus 250g H_2O pot) whenever the plants began to wilt. At day thirteen, the salt treatment pots received 4g/pot NaCl dissolved in the irrigation water. At the two subsequent waterings, 4g/pot of NaCl was added to each of the pots being subjected to higher salt treatments, until the final concentrations were reached. These final concentrations (0, 4, 8 and 12g/pot) resulted in the soils having salinity levels of 0.26, 0.45, 0.64 and 0.83 dS/m $EC_{1:5}$ respectively, which was approximately equivalent to 2.6, 4.5, 6.4 and 8.3 dS/m ECe.

At harvest, the number of grains and total grain weight were recorded for each pot. Analysis of variance was conducted using Genstat 6®.

4.4.3 Results

Analysis of variance revealed that the salt treatments had a significant effect on the total grain weight ($P<0.001$) (Figure 4.2), but that variety had no significant effect.

Figure 4.2. The effect of four levels of soil salinity (dS/m $EC_{1:5}$) on the mean grain yield/pot of two wheat varieties (Worrakatta and Krichauff) grown in 250mm pots in a glasshouse.



Similarly, the salinity treatments also had a significant effect ($P<0.001$) on the number of grains produced per pot (Figure 4.3), while there was no significant effect of genotype.

The interaction between salinity and genotype had a significant ($P<0.001$) effect on mean seed weight (Figure 4.4 and Plate 4.1). This interaction resulted in the seed weight of Worrakatta being reduced to a greater extent (16.4%) in response to increased salinity than that of Krichauff (7.7%) across the range of salinity tested (0.26-0.83 dS/m), despite Worrakatta having a heavier mean seed weight when grown in the soil with the two lowest salinity treatments (0.26 and 0.45 dS/m).

Figure 4.3. The effect of four levels of soil salinity (dS/m $EC_{1:5}$) on the mean number of wheat grains (mean of Worrakatta and Krichauff) produced per pot (total of four plants).

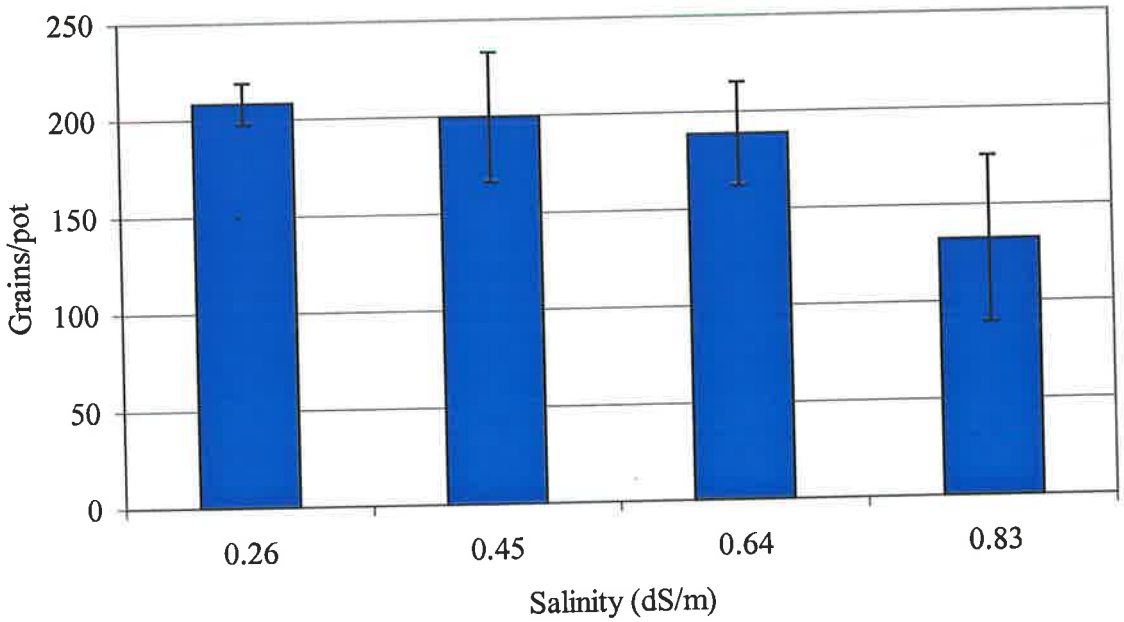


Figure 4.4. The effect of four levels of soil salinity (dS/m $EC_{1:5}$) on the mean seed weight (mg) of the wheat varieties Worrakatta and Krichauff.

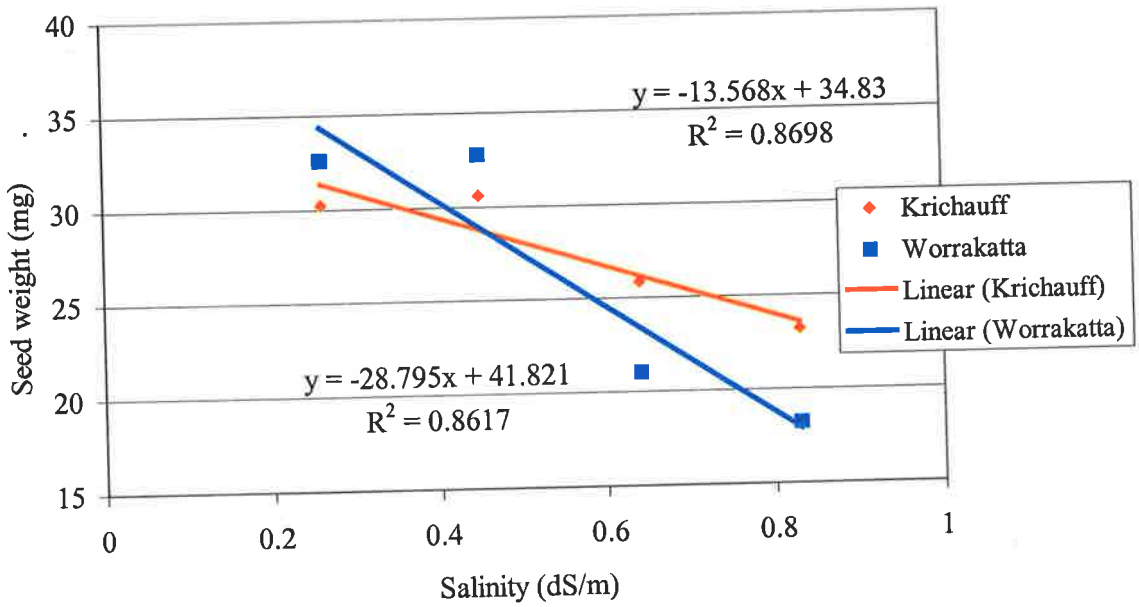
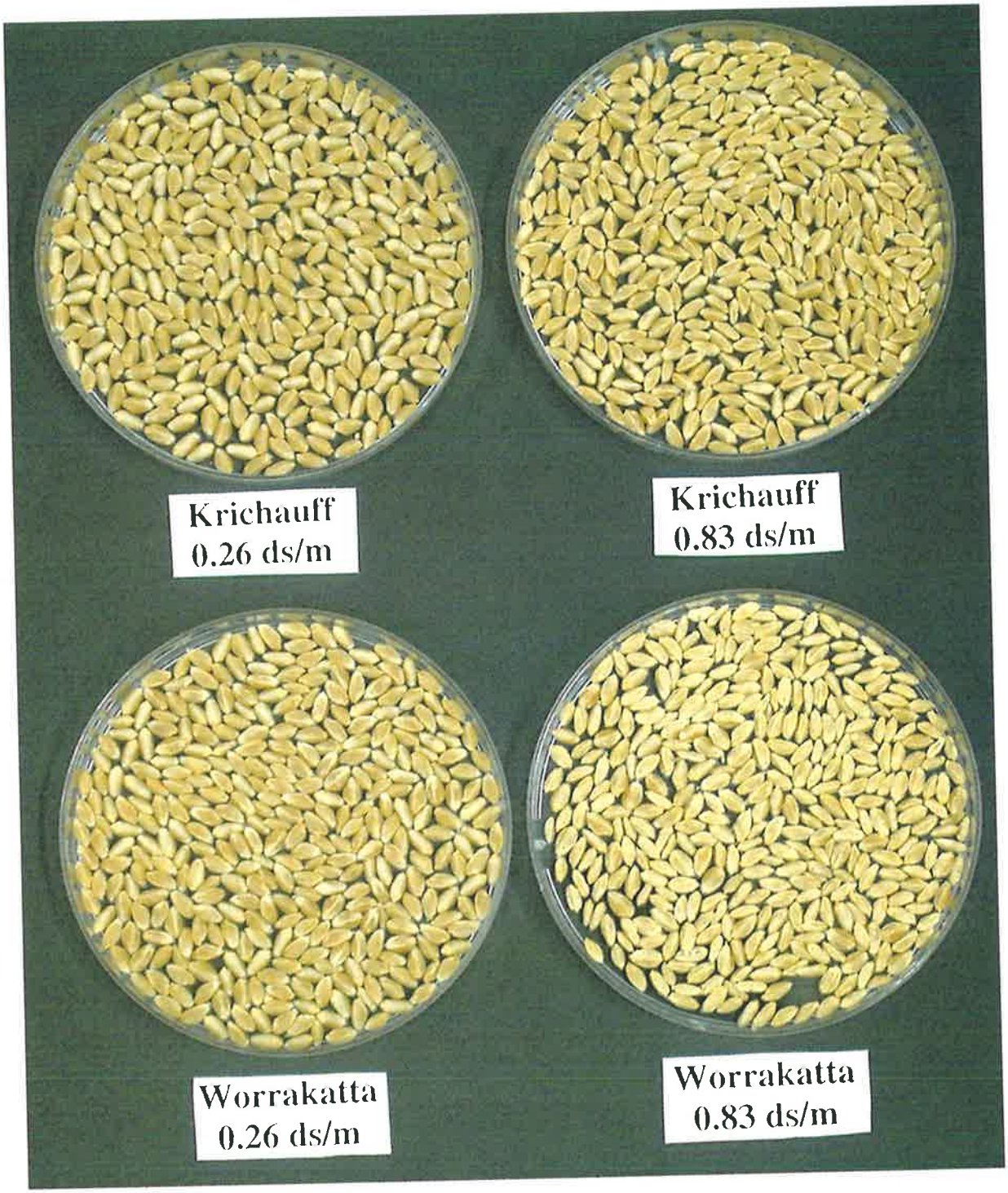


Plate 4.1. The effect of salinity on the grain size and appearance of the sister varieties Worrakatta and Krichauff grown in pots in the glasshouse.



4.4.4 Discussion

Salinity had a large and significant effect on grain yield and the number of grains produced, but no statistically significant interaction was detected. Grain size on the other hand, was affected by the interaction between salinity and genotype, with the grain size of Worrakatta being reduced to a greater extent than that of Krichauff in response to increasing salinity (16.4% compared with 7.7%), suggesting that the higher percentage of screenings commonly measured in Worrakatta was a result of its response to salinity. While Na concentration in shoots was not measured in this experiment, it has been shown that the Na accumulation of Worrakatta was significantly higher, both in pots (Section 4.2) and in the field (Section 4.5). Presumably, the higher Na accumulation of Worrakatta resulted in reduced photosynthetic capacity during grain fill and consequently, smaller grains.

4.5 Field testing of the $F_{2,5}$ derived lines to assess the relationship between Na exclusion and transient salinity tolerance.

4.5.1 Introduction

The toxic effects of elevated levels of Na and Cl ions on metabolism have been investigated by many researchers (Flowers, *et al.*, 1977; Greenway *et al.*, 1981; Jennings, 1976). From this evidence Greenway and Munns (1980) drew the conclusion that reduced uptake, or active exclusion of these ions by plants should improve plant tolerance. However, direct evidence that Na exclusion confers field tolerance to salinity has not been reported in the literature (Section 7.1).

Dvorak *et. al.* (1994) have shown that the addition of the *Kna1* locus on chromosome 4D of bread wheat to the durum genome reduced Na accumulation. Field testing of 25 translocation lines showed that those which contained the *Kna1* locus had significantly higher biomass production than those that did not. Further evidence that Na exclusion improved tolerance has been provided by the higher grain yield and reduced screenings achieved by durum lines carrying a Na exclusion gene at Two Wells in 2003 (Chapter 7).

A series of field experiment was undertaken to assess whether the difference in Na exclusion segregating in this bread wheat population had a significant effect on grain yield. These experiments were located on sites affected by transient salinity at Buckleboo, Port Pirie, Redhill and Two Wells in 2003. Grain size (% passing through a 2.2mm screen) was also measured to check whether the variation in grain size within the population was correlated with, or independent of Na exclusion.

4.5.2 Materials and methods

Seed

Of the $F_{2,5}$ derived F_7 lines sown in the field experiment in 2002, only 141 (representing fifty-four of the original sixty F_2 derived families) produced enough seed to be sown in non-replicated experiments at four locations (Port Pirie, Redhill, Two Wells and Buckleboo) in 2003. The seed was cleaned through a Carter-Day® Dockage Tester, to remove chaff and seed with a grain size smaller than 2.2mm.

Experimental Design

At each site, the 141 $F_{2,5}$ derived lines were sown (non-replicated) in 4 row x 4.2m plots in the standard method employed by the Waite durum breeding program (Chapter 3). Also included, were twenty-four plots of the high Na accumulating parent, Worrakatta, which along with the $F_{2,5}$ derived lines, were randomized within the experiment. Krichauff check plots were included every fifth plot throughout the experiment (total of forty-five), starting in column one of odd numbered bays and column three in even numbered bays. The total number of plots was 210 arranged into fifteen bays and fourteen columns. The Two Wells experiment was sown on the fourth of June, followed by Buckleboo on the tenth and both Redhill and Port Pirie on the eleventh of June.

Prior to seeding each trial site was sprayed with 1.2L/ha of trifluralin and 1L/ha of glyphosate. All plots received DAP fertiliser (N:P:K:S 18:20:0:0) at seeding at a rate of 60kg/ha. Post-emergent herbicides were applied by the farmers at each location as required.

Na concentration of whole tillers sampled from Redhill

On the twenty-seventh of September, twelve whole tillers were sampled at random from every plot in the Redhill experiment, dried at 80°C for twenty-four hours, ground in a stainless steel mill and passed through a 2mm screen. The samples were digested in nitric acid and analysed by ICP-spectrometry by Waite Analytical Services. The Worrakatta and Krichauff plots were analysed initially to ensure that they were significantly different, before proceeding with the analysis of the $F_{2,5}$ derived lines. The mean Na concentration of the two ICP-spectrometry runs could be different, due to slight differences in conditions.

Grain yield and screenings

After maturity the plots were mechanically harvested. The Buckleboo site was harvested on November 21, 2003, followed by Port Pirie, Redhill and Two Wells, on November 24, and the second and fifth of December respectively. The grain samples were weighed and cleaned through a Carter-Day® Dockage Tester using a 2mm screen. The portion of grain passing through the screen was weighed and the screenings percentage calculated.

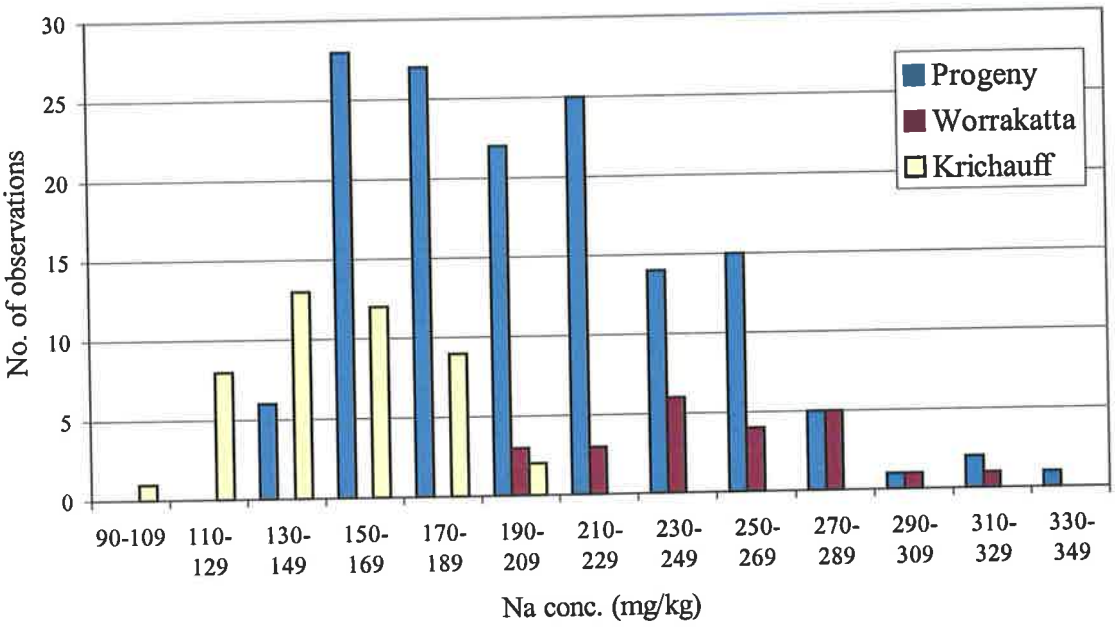
4.5.3 Results

Na concentration of whole tillers sampled from Redhill

The mean Na concentration in the whole tillers sampled from the Worrakatta and Krichauff plots were 238 ±52 and 151 ±23mg/kg respectively. The data was analysed in Genstat (Edition 6) using the Mann-Whitney 'U' test, which indicated that the Na concentration of Worrakatta was significantly higher than that of Krichauff ($P<0.01$). There was very little overlap in the distributions of the Na concentration in the whole tops of Worrakatta and Krichauff, and no evidence of transgressive segregation, so that the $F_{2,5}$ derived lines with Na concentrations approaching the extremes of the distribution (Figure 4.5) could be regarded as having parental genotypes.

The mean concentration of Na in the tillers of the $F_{2,5}$ derived lines was 208 ±61mg/kg, which was intermediate to the mean Na concentrations of the two parents. The range of Na concentration observed in the progeny was 135-350 mg/kg, with an outlier at 750mg/kg, which has been removed because it was probably due to contamination (Figure 4.5).

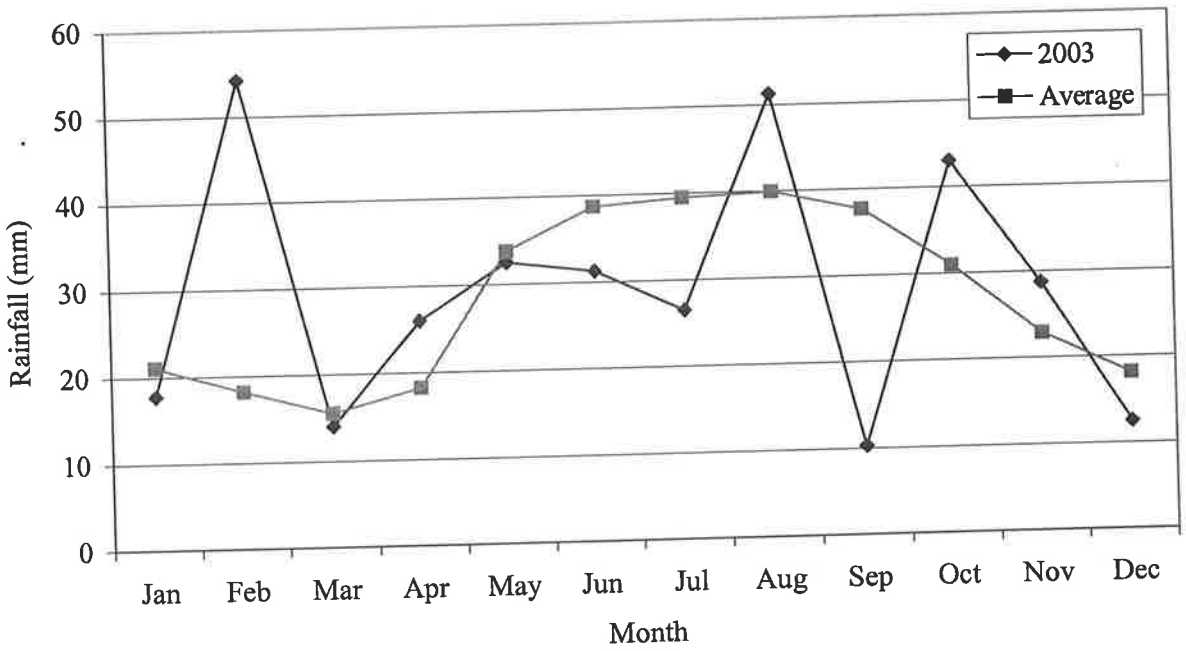
Figure 4.5. Distribution of 141 $F_{2,5}$ derived lines from the cross (Worrakatta*Krichauff) for mean Na concentration (mg/kg) in twelve whole tillers, compared to the distributions of Worrakatta (n=24) and Krichauff (n=45), sampled at Redhill, September 2003.



Grain yield

The cereal growing districts north of Adelaide and on the northern Eyre Peninsula, received below average rainfall in September and October, 2003 and very high temperatures on the sixteenth of November. This was likely to have increased the reliance on subsoil moisture during grain fill and should have increased any benefit of tolerance to subsoil toxicities such as transient salinity. The comparison between 2003 and longterm average rainfall is displayed in Figure 7.2 (see Chapter 7) for the Two Wells, Redhill and Port Pirie locations. While the total October rainfall at Buckleboo of 43mm was above the average rainfall of 31mm (Figure 4.6.), the crop was severely drought affected during September (Ramsey pers comm.).

Figure 4.6. Total monthly rainfall (mm) recorded in 2003*, compared to long-term average** (n=35) data for Buckleboo.



* data kindly provided by Mr. Rowan Ramsey and; ** the Australian Bureau of Meteorology.

The mean grain yield of the Worrakatta, Krichauff and the $F_{2,5}$ derived progeny are tabulated in Table 4.3.

Table 4.3. Mean grain yields (g/plot) at four locations for Worrakatta, Krichauff and the $F_{2,5}$ derived lines from the (Worrakatta*Krichauff) cross, 2003.

	Worrakatta	Krichauff	$F_{2,5}$ derived lines
Redhill	778 ±146	762 ±131	807 ±147
Buckleboo	290 ±31	289 ±27	285 ±37
Two Wells	917 ±142	930 ±128	943 ±155
Port Pirie	223 ±104	213 ±134	206 ±112
Mean	552	549	560

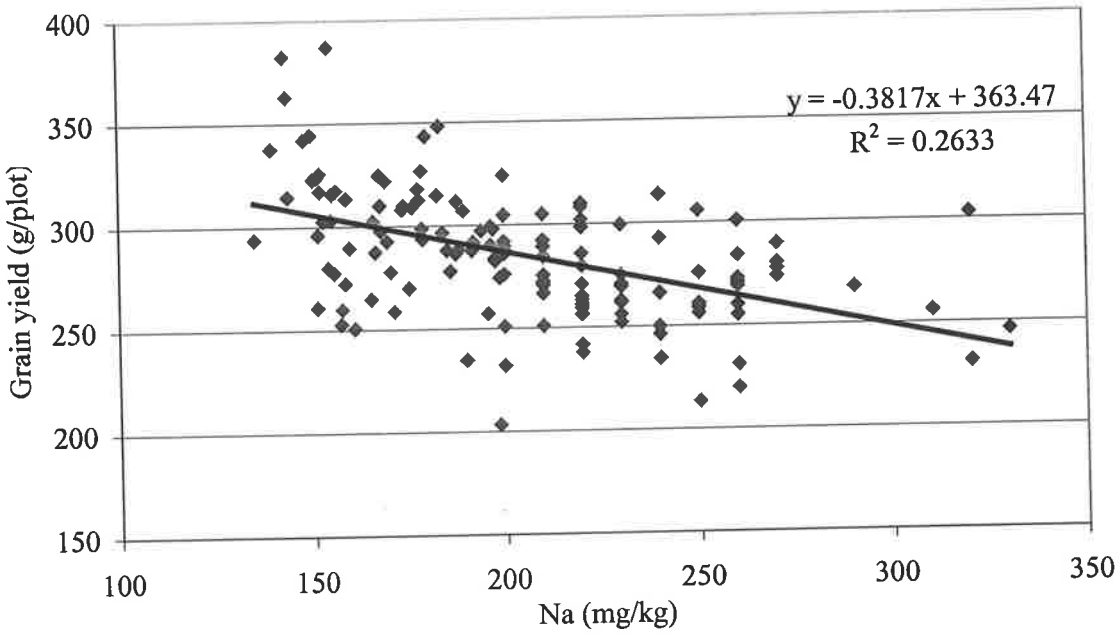
The grain yield of the $F_{2,5}$ derived lines were compared across sites and with the Na concentration measured in the whole tillers sampled from Redhill (Table 4.4). Buckleboo was the only site where grain yield was significantly correlated with the Na concentration in the whole tillers sampled from Redhill (Figure 4.7). The grain yield at Buckleboo was not correlated with any other location, providing further evidence that Na exclusion had no significant impact on the grain yields measured at the other locations. The only significant correlation between sites was between Port Pirie and Redhill, neither of which were correlated with Na concentration. This indicated that another trait(s) was segregating within this population, affecting grain yield.

Table 4.4. Correlation matrix for the grain yields at four locations in 2003 and Na concentration in the whole tillers sampled from Redhill for the $F_{2,5}$ derived lines from the cross (Worrakatta*Krichauff).

	Buckleboo	Port Pirie	Two Wells	Redhill
Na (Redhill)	-0.51***	-0.05 ns*	-0.01 ns	0.00 ns
Buckleboo		-0.05 ns	-0.04 ns	-0.01 ns
Port Pirie			0.01 ns	0.19*
Two Wells				0.08 ns
Redhill				

* not significant; * (P<0.05); ** (P<0.01); *** (P<0.001)

Figure 4.7. The relationship between Na concentration in whole tillers sampled at Redhill and grain yield at Buckleboo, 2003 for the $F_{2,5}$ derived lines from the cross (Worrakatta*Krichauff).



Screenings

Worrakatta and Krichauff had significantly different screenings percentages at Redhill ($P<0.05$), Two Wells ($P<0.01$) and Port Pirie ($P<0.05$) on the basis of the Mann-Whitney 'U' test (Table 4.5).

Table 4.5. Screenings (%) in grain harvested at four locations of Worrakatta, Krichauff and the $F_{2,5}$ derived lines from the (Worrakatta*Krichauff) cross, 2003.

	Worrakatta	Krichauff	$F_{2,5}$ derived lines
Redhill	12.0 ±2.0	9.0 ±1.6	10.5 ±2.7
Buckleboo	7.6 ±1.6	7.3 ±1.6	7.3 ±1.6
Two Wells	22.1 ±5.4	13.6 ±4.1	16.8 ±5.2
Port Pirie	25.5 ±3.6	20.0 ±4.2	22.7 ±5.4
Mean	16.8	12.5	14.3

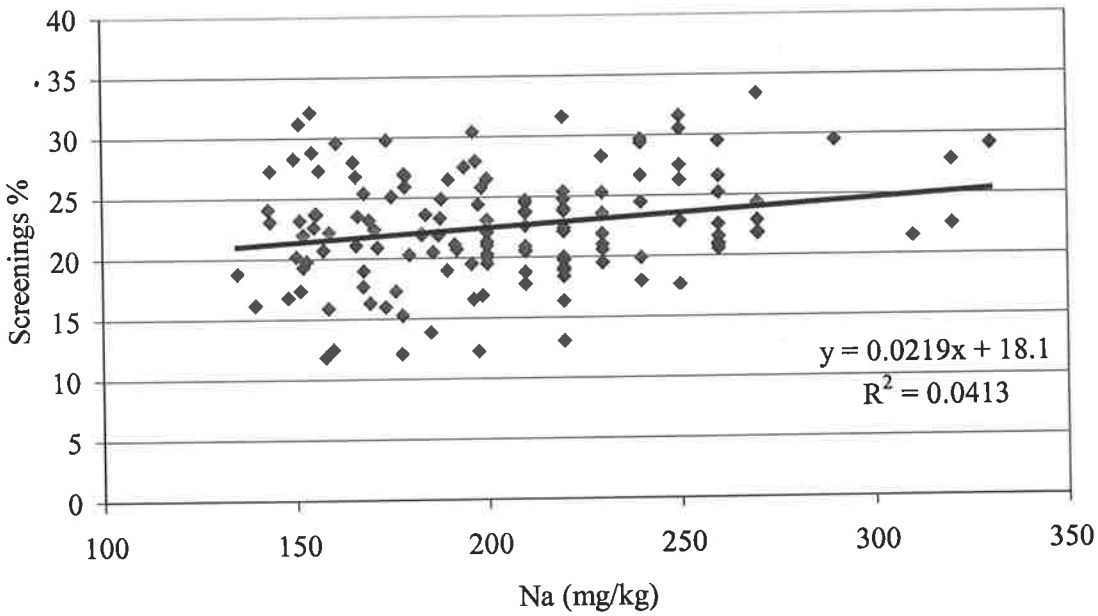
When the screenings percentages of the $F_{2,5}$ derived lines were correlated across sites and with the Na concentration measured in the whole tillers sampled from Redhill (Table 4.6), the only significant correlation was with the Port Pirie experiment (Figure 4.8).

Table 4.6. Correlation coefficients for the comparison of screenings percentages between sites and with Na concentration in whole tillers sampled at Redhill, 2003.

	Redhill	Buckleboo	Two Wells	Port Pirie
Na (Redhill)	0.11 ns*	0.14 ns	0.13 ns	0.20*
Redhill		0.23**	0.26**	0.24**
Buckleboo			0.31***	0.34***
Two Wells				0.44***
Port Pirie				

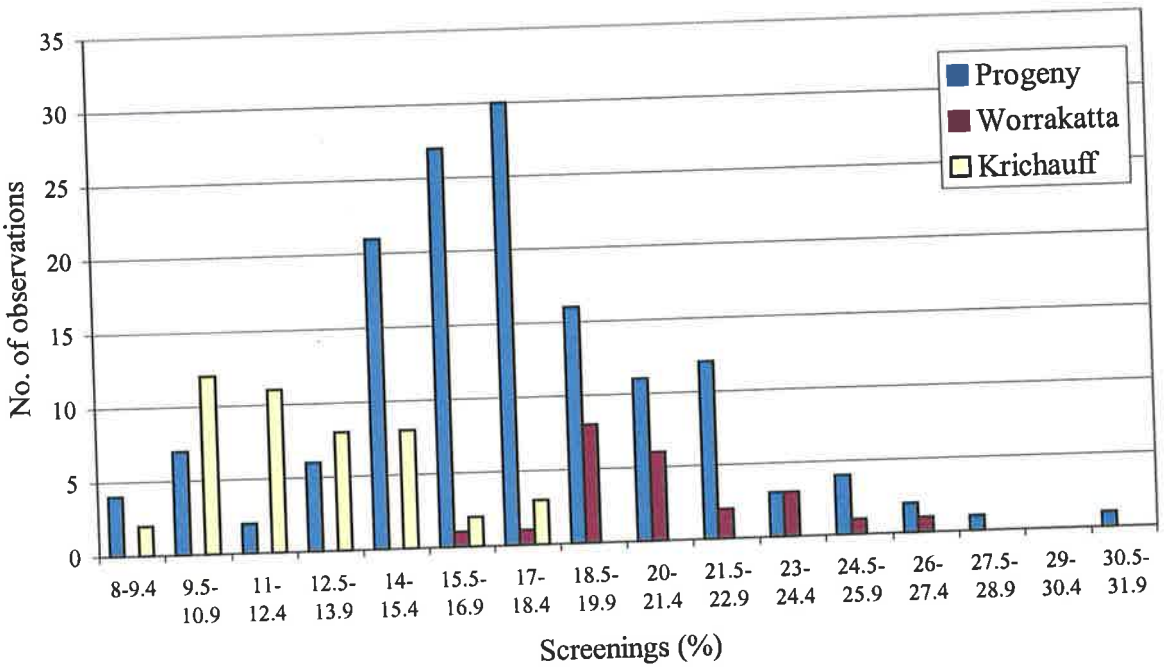
* not significant; * (P<0.05); ** (P<0.01); *** (P<0.001)

Figure 4.8. The relationship between Na concentration (mg/kg) in whole tillers sampled at Redhill and screenings percentage at Port Pirie for $F_{2,5}$ derived lines from the cross (Worrakatta*Krichauff).



The screenings percentages in the grain harvested from the $F_{2,5}$ derived plots at Port Pirie were strongly correlated with Redhill ($P<0.01$), Two Wells ($P<0.001$) and Buckleboo ($P<0.001$). This suggests that there were other genetic factors affecting the level of screenings in this population, which were not related to Na exclusion. Of these sites, Two Wells had the highest mean screenings percentage and the largest difference between Worrakatta and Krichauff. If an inherent difference in grain size occurs between Krichauff and Worrakatta, segregation should be observable in the $F_{2,5}$ derived lines grown at Two Wells (Figure 4.9).

Figure 4.9. The distribution of the screenings percentage at Two Wells (2003) of the $F_{2,5}$ derived lines from the cross (Worrakatta*Krichauff) compared to the parental varieties.



The distributions of the two parental varieties are almost disjunct and distinct, while the variance of the $F_{2,5}$ derived lines is much greater and suggestive of the segregation of two or

more genes. As the range of the distribution of the progeny extends somewhat beyond that of the two parents, there is some suggestion of transgressive segregation.

4.5.4 Discussion

The Na concentrations in whole tillers of Worrakatta and Krichauff were significantly different at Redhill, despite the concentrations being lower than expected, as whole tillers taken from locations with similar soil Na^+ levels typically have Na concentrations twice that observed at Redhill. The small overlap between in the distributions of Worrakatta and Krichauff, allows confident classification of the $F_{2,5}$ derived lines at the extremes of the distribution as being of the parental genotype (Figure 4.5).

The distribution of the Na concentration of the $F_{2,5}$ derived progeny is not incompatible with control by a single locus, as there is no evidence of transgressive segregation and there are a high proportion of parental types rather than a high proportion intermediate to the two parents (Figure 4.5). One option to verify the genetic control is to perform a bulked segregant analysis using lines selected from the extremes of the distribution. If successful, this method would elucidate the number and location of the loci controlling the Na^+ exclusion trait.

The only positive correlations with the Na concentration at Redhill, were with the grain yield at Buckleboo and screenings at Port Pirie. The Buckleboo site suffered severely from drought during September, before a substantial rain of 40mm over the first and second days of October, enhanced grain filling. Apparently the yield of the lines accumulating more Na had already been limited to a greater extent than that of the Na excluders.

The Port Pirie site did not receive rain to relieve the spring drought until after maturity. Consequently, the grain size was much smaller than that at Buckleboo, and the grain size of the higher Na accumulators was more severely affected than that of the Na excluders. Even if the number of grains was determined before any difference in Na exclusion had an effect, a correlation with Na accumulation would be expected to occur through a correlation with the size of individual grains (ie. higher screenings percentage with the same number of grains should result in lower yield). As this was not observed, the only conclusion was that the measurement of yield was less accurate than that of grain size, due to spurious variation arising from the presence of crown rot and perhaps errors in the management and harvesting of plots.

A large portion of the variation in screenings among the $F_{2,5}$ derived progeny did not appear to be the result of the variation in Na exclusion. At three of the four sites, there was a significant difference between the two parents, yet a correlation between Na exclusion and screenings only occurred at one of these sites. The three sites where a correlation between Na concentration and screenings did not occur were all correlated with each other, suggesting that there is segregation for grain size, independent of Na exclusion.

The variation in grain size in this population provides the opportunity to identify gene/s that could be of value to breeding programs. The distribution of screenings was studied at the Two Wells site because there was a large difference between Worrakatta and Krichauff (22.1 and 13.6% screenings respectively), yet the screenings percentages of the $F_{2,5}$ derived progeny was not significantly correlated with Na concentration at Redhill. While it was not possible to identify the number of loci controlling the trait from the unreplicated Two Wells data,

collating data from similar sites in the future may allow the progeny to be separated into defined classes of segregants, allowing conclusions about the genetic control of the trait to be drawn. Also, if the trait is controlled by more than one loci and transgressive segregants were to be detected, it may be possible to select a genotype with grain size stability superior to that of Krichauff.

4.6 Chromosomal location of the Na exclusion locus of Krichauff determined by bulked segregant analysis.

4.6.1 Introduction

The ability of Krichauff to exclude more Na than other genotypes may be an important adaptational character for soils affected by transient salinity. While the 2003 field experiments only identified a significant correlation between Na accumulation and grain yield at one site of the four sites studied, further field experiments to be conducted in 2004 will provide more information on the value of the trait. If the additional Na excluding ability of Krichauff is proved to be of agronomic value, a closely linked molecular marker could be a more efficient way to select for the locus than either the glasshouse screening or sampling field plots for Na analysis by ICP spectrometry. While the cost of screening genotypes using ICP spectrometry or molecular markers is not dissimilar, non-genetic effects on uptake can result in inaccuracy of categorisation on the basis of the ICP-spectrometry analysis.

It was possible that this trait in Krichauff was homoeologous to either the *Kna1* locus on 4DL of bread wheat (Dubcovsky *et al.*, 1996), or the QTL on chromosome 2A of the durum landrace, Na49 (Munns *et al.*, 2003). If this were the case, it could have been expected to have

an effect of similar magnitude to these loci and provide an alternative genetic mechanism for improving the salt tolerance of durum wheat. While work is currently underway to incorporate the Na exclusion of Na49 into the genetic background of the adapted variety Kalka (Chapter 5), and Dvorak (1994) attempted to incorporate *Kna1* into the background of Langdon by homoeologous recombination, the success of both of these programs may be limited by linkage drag, or the disruption of linkage blocks of importance to commercial durum production. Under these circumstances, pursuing this option from the bread wheat, Krichauff, may provide a useful alternative strategy.

4.6.2 Material and methods

Selection of lines for bulked segregant analysis

The Na data obtained from the Redhill field plots (Figure 4.5) was analysed spatially, but because the raw data exhibited better separation of the distributions of the Na concentrations of Worrakatta and Krichauff than those from any of the spatial adjustments, initially the unadjusted data was used to select the two sets of $F_{2,5}$ derived progeny from the extremes of the distribution.

Most of the variation in Na concentration arising from environmental effects appeared to be between bays, indicating that variation along each bay was minimal and primarily due to genetic effects. Consequently the data was also expressed relative to the moving mean of the eight neighboring plots. This was undertaken using customized software written at the University of Adelaide, which was normally used for expressing the grain yield of plots as a percentage of the four plots on either side.

The moving mean adjusted data was then examined to remove any lines selected on the basis of the raw data that may have been included primarily because of environmental variation. From these two groups of lines (high and low Na), eleven low Na lines and twelve high Na lines were retained which did not include a single F_2 family more than once in each group (Table 4.7).

F_5 derived F_6 seed of each of the selected lines were provided to Drs. J.P.E. Cheong and K.J. Williams (CRC for Molecular Plant Breeding) to undertake bulked segregant analysis using the procedures described in Cheong *et al.* (2004) and Wallwork *et al.* (2004).

Table 4.7. $F_{2,5}$ derived progeny of the cross (Worrakatta*Krichauff) selected for high and low Na accumulation on the basis of adjusted and unadjusted Na concentration in 12 whole tillers from Redhill, 2003. The mean concentrations of Krichauff and Worrakatta were 238 ±52 and 151 ±23 mg/kg of Na respectively.

F_2 family	F_5 reselection	Na Conc. (raw)	Na Conc. (mm8)*	Na classificaltion
10	2	150	148	Low
14	4	135	158	Low
27	4	155	158	Low
30	3	155	159	Low
38	1	156	168	Low
39	3	144	153	Low
43	3	151	167	Low
44	3	143	140	Low
46	1	153	160	Low
47	3	151	162	Low
51	2	144	165	Low
4	2	270	268	High
5	4	250	279	High
16	1	260	250	High
22	4	270	268	High
27	2	260	259	High
30	4	250	255	High
33	1	330	288	High
39	1	260	277	High
41	2	270	258	High
42	4	320	306	High
45	1	310	295	High
48	2	260	289	High

* mm8 data adjusted proportional to the mean of the 8 nearest plots, relative to the grand mean.

4.6.3 Results

The Na excluding and non-excluding bulks, along with the parental varieties, were analysed with 144 primer combinations that allowed screening of approximately 800 AFLP loci. Of these, twenty-five were identified as possible candidate markers. The twenty-three F_5 derived lines were individually scored for these AFLP fragments, of which nine markers had linkage greater than 70%. The chromosomal location of two AFLPs, *PstATA-MseCTC (134)* and *PstACC-MseCGC (198)*, which were both 78% linked to the trait, were mapped in the CD87/Katepwa mapping population. Both of these were found to be located on chromosome 4B.

To confirm the accuracy of the AFLP mapping and the chromosome 4B location of the Na exclusion locus, simple sequence repeat (SSR) microsatellite markers (*gwm149* and *gwm513*) of this region (developed by Röder *et al.*, 1998), were genotyped on the twenty-three selected (Worrakatta*Krichauff) lines, but only *gwm149* was polymorphic. Together with the two AFLP markers, the SSR marker explained up to 61% of the total phenotypic variation (Table 4.8), confirming that the Na exclusion locus is located on the long arm of chromosome 4B (Figure 4.10). This percentage would be less if all 141 lines in the population had been included in the analysis, as the genotyped lines were selected from the extremes of the distribution (Figure 4.5) to reduce the likelihood of incorrect categorisation of the lines in each bulk.

The SSR marker *gwm149* is also linked to *Rht1*, so the lines were also genotyped for *Rht1* to ensure that the Na exclusion trait was controlled by an independent locus. All twenty-three

lines had a positive band for *rht1-mutant*, indicating that the trait is not pleiotropic to the effect of *Rht1* and is attributable to a separate locus.

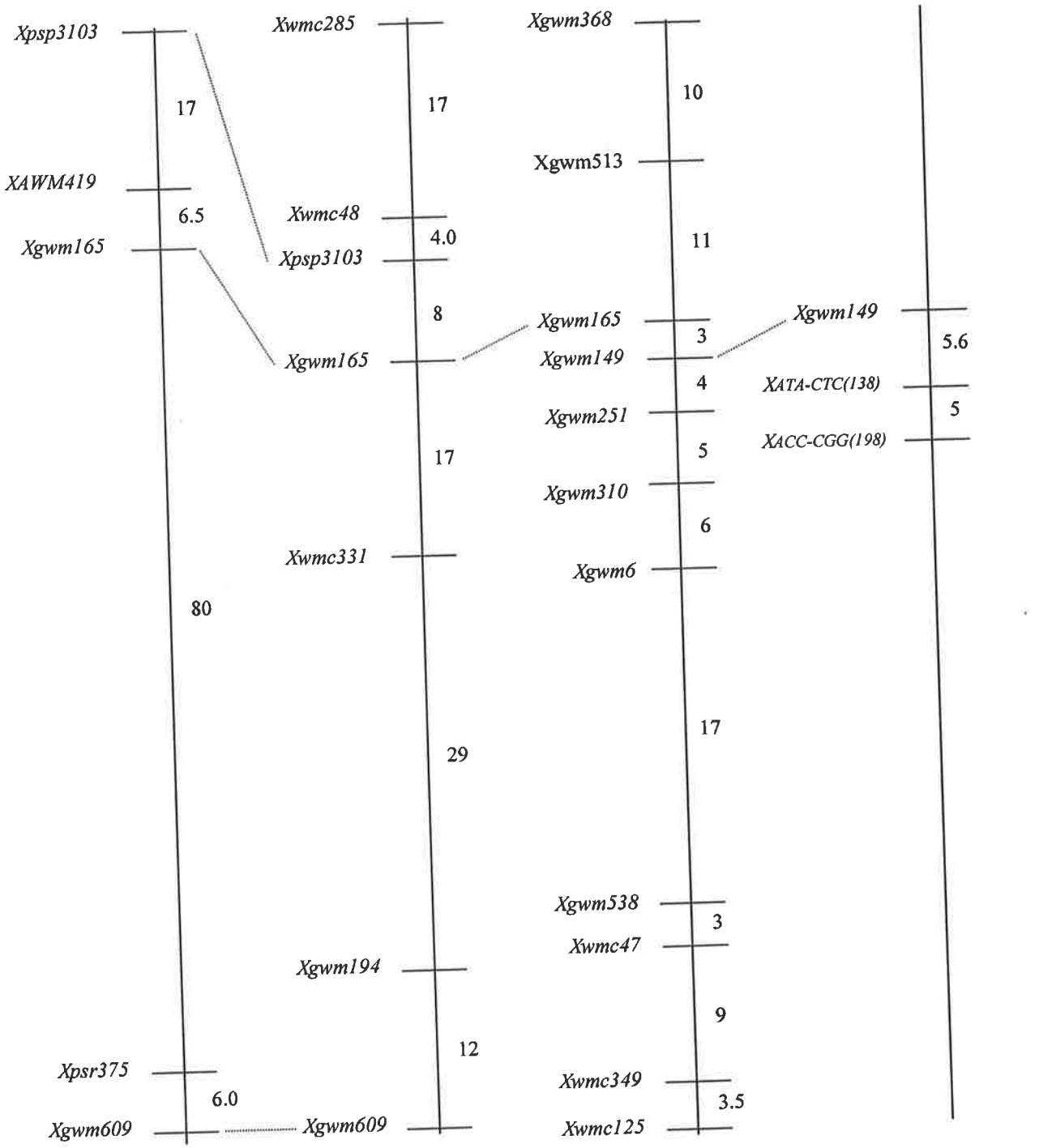
The *Kna1* locus identified by Dubcovsky *et al.* (1996) was completely linked to the RFLP, *Xpsr375* on chromosome 4D. This marker is approximately 70cM distal to the *gwm149* marker, as shown by the partial map of chromosome 4D of the CS/DH* mapping population (Figure 4.10). This indicates that *Kna1* is not homoeologous to the Na exclusion locus located in chromosome 4B of Krichauff.

Table 4.8. Genetic distance between AFLP and SSR markers linked to a Na exclusion trait on the long arm of chromosome 4B of Krichauff.

Distance	Marker interval	LOD score	R^2
	Gwm149		
5.6cM		4.64	61
	ATA-CTC		
5.0cM		2.34	37
	ACC-CGG		

* The CS/DH map is based on as yet unpublished data of S. Quarrie. See Appels, (2003).

Figure 4.10. (a) Partial map of chromosome 4D of the CS/DH cross, showing the mapping of the *Kna1* linked marker *Xpsr375*, relative to *Xgwm165*. *Xgwm165* is also located on chromosomes 4D (b) and 4B (c) of the W7976/Opata85 cross. This marker is also closely linked with *Xgwm149* on chromosome 4B of the W7976/Opata85 cross, which is closely linked with the Na exclusion locus on 4B of Krichauff (d).


(a) CS /DH (4D)
(b) W7976 /Opata85 (4D)
(c) W7976 /Opata85 (4B)
(d) Worrakatta /Krichauff (4B)
Xpsp3103
17
XAWM419
6.5
Xgwm165
80
Xpsr375
6.0
Xgwm609
Xwmc285
17
Xwmc48
4.0
Xpsp3103
8
Xgwm165
17
Xwmc331
29
Xgwm194
12
Xgwm609
Xgwm368
10
Xgwm513
11
Xgwm165
3
Xgwm149
4
Xgwm251
5
Xgwm310
6
Xgwm6
17
Xgwm538
3
Xwmc47
9
Xwmc349
3.5
Xwmc125
Xgwm149
5.6
XATA-CTC(138)
5
XACC-CGG(198)

4.6.4 Discussion

The extra Na excluding ability of Krichauff is controlled by a single locus located on the long arm of chromosome 4B. This locus co-segregated with the microsatellite marker *gwm149* in 91.3% (21) of the twenty-three lines included in the analysis. This marker was mapped by Röder *et al.* (1998) to a region of the long arm of chromosome 4B, between 11.7 and 15.2cM from the centromere. Hence it is highly unlikely that this locus is homoeologous to the *Kna1* locus identified by Dubcovsky *et al.* (1999), which is located approximately 70cM distal to *gwm149* (Figure 4.10). Likewise, it cannot be homoeologous to the Na exclusion locus of Na49, which is located on chromosome 2A (Munns *et al.*, 2003).

The *gwm149* marker needs to be genotyped in all of the $F_{2,5}$ derived lines to obtain a more accurate assessment of the percentage of variation for which it accounts, as well as the frequency of recombination between the marker and the Na exclusion locus. To do this effectively, a more accurate assessment of the Na accumulation of each line will need to be undertaken in another experiment to reduce the environmental variation present in the Redhill data. While this work is still to be carried out, the indications are that *gwm149* and the Na exclusion phenotype are linked sufficiently close for the marker to be used as a selection tool.

4.7 Discussion of Chapter 4.

The difference in the level of Na exclusion between Worrakatta and Krichauff has been verified in a pot experiment in the glasshouse. This difference in Na exclusion was not detectable at low NaCl concentrations, but when the varieties were grown in substrate containing a high concentration of NaCl, the two varieties were significantly different in Na uptake (Table 4.2).

A similar glasshouse experiment showed that the fifty-seven F_2 derived lines from the cross (Worrakatta*Krichauff) were segregating for the Na exclusion trait. Unfortunately, the variation within the experiment did not allow an accurate classification of the lines into distinct classes (Figure 4.1). These lines were reselected in the F_5 generation, to produce a population of 228 lines.

These $F_{2,5}$ derived progeny were grown at four transient saline sites in 2003, to measure any benefit conferred by the Na exclusion trait. Of the four sites, the Na concentration in whole tillers (sampled at Redhill) was only correlated with grain yield at Buckleboo (Figure 4.7) and with the percentage of screenings at Port Pirie (Figure 4.8). Both of these sites were affected by a severely dry September, suggesting that the Na exclusion of Krichauff only provides a benefit under severe drought conditions on saline sites. Further experiments conducted over more locations and years are needed to elucidate more detailed information on the magnitude of the yield benefit and the soil and climatic conditions where this occurs.

Bulked segregant analysis was performed using $F_{2,5}$ derived lines selected from the extremes of the distribution for Na concentration at Redhill (Figure 4.5). This analysis revealed that the ability of Krichauff to exclude more Na from the above ground parts of the plant than Worrakatta is controlled by a locus on the long arm of chromosome 4B, which is linked to the SSR marker *gwm149*. This marker explained 61% of the variation in Na concentration among the 23 lines that comprised the bulks.

One of the principal aims of this work was to determine whether the high screenings percentage of Worrakatta resulted from it accumulating more Na than Krichauff. The pot experiment undertaken to investigate the relationship between variety (Worrakatta and Krichauff) and soil salinity (Section 4.4), detected a significant (genotype x salinity) interaction, which supported the hypothesis that the higher Na accumulation of Worrakatta resulted in smaller grain when grown in saline soil. While this result was reflected in a correlation between Na uptake and screenings at Port Pirie, the large variation in screenings observed at other sites was not correlated with Na uptake. This additional variation was correlated across sites and suggested that another grain size related trait was also segregating in the population, despite the very close genetic relationship between the two varieties.

Chapter 5.

INTROGRESSION OF A SODIUM EXCLUSION TRAIT INTO DURUM WHEAT AND ITS MODE OF INHERITANCE

5.1 Introduction

Breeding crop varieties with improved salt tolerance is an attractive option for improving production on soils affected by transient salinity. In South Australia, mapping work led by David Maschmedt of PIRSA Land Information (2001) estimated that a large proportion of the cropping area has subsoil salinity levels in excess of 4 dS/m ECe (Figure 2.1). Maas and Hoffman (1977), after reviewing the world literature on salt tolerance of plants, concluded that the normal response to salinity was a threshold level, above which there was a linear reduction in yield. The threshold for moderately sensitive crops such as wheat was found to be approximately 4dS/m ECe.

The current major areas of durum production in South Australia are the Adelaide Plains, northern Mt. Lofty Ranges and northern York Peninsula. These regions are dominated by subsoils of less than 4 dS/m ECe (Figure 2.1). Other major cropping regions that could be suitable for producing high quality durum, such as the Eyre Peninsula, Murray Mallee and the coastal plains stretching from Two Wells, through Brinkworth, to Port Pirie are dominated by subsoils with salt concentrations of 4dS/m and above. As other subsoil constraints also occur in these soils, it is likely that breeding for better tolerance for all these and reduced Na uptake will have a large impact on grain production.

Despite the world-wide interest in salt tolerance, the release of crop varieties with improved salt tolerance has been rare. In a review of literature, Noble and Rogers (1992)

found only six crop varieties that had been developed specifically for improved salt tolerance. These included a variety each of avocado (Cooper, 1951), wheatgrass (Dewey, 1962), rice, (Gad El Hak, 1966), alfalfa (Dobrenz *et al.*, 1983), red fescue (Cordukes, 1981) and maize (Day, 1987). These have all been selected on the basis of yield or survival under saline conditions (tolerance per se), except the alfalfa variety, which was selected on the basis of its ability to germinate in saline solution.

Screening for tolerance per se requires extensive field-testing to validate laboratory results. This can be expensive, slow, imprecise and restrictive on the number of lines that can be screened. A more efficient approach is to establish that a specific trait is an important adaptational character in the target environment and then select lines on the basis of a laboratory test. This selection must occur under conditions which have both a high heritability and are closely correlated with the target environment.

Genotypes that are able to exclude Na from the roots are expected to have greater tolerance to saline soils through a reduction in leaf damage due to an excess of ions (Munns *et al.*, 2000). As reported by Shah *et al.* (1987), commercial durum wheat varieties (*Triticum turgidum* L. *ssp. durum*) accumulate much larger amounts of Na in plant tops than bread wheat (*Triticum aestivum* L.). It has also been noted that the durums are less salt tolerant (Francois *et al.* 1986; Rawson *et al.* 1988). Francois *et al.* (1986) applied irrigation treatments with varying salinity levels and measured the effect on the grain yield of one bread wheat and two durum cultivars. It was found that grain yield of the bread wheat and durum was unaffected by soil salinity up to 8.6 and 5.9 dS/m (ECe), but that each unit increase in salinity above these thresholds reduced yield by 3.0% and 3.8% respectively. Rawson *et al.* (1988) screened a selection of barley, triticale, durum and bread wheat

varieties in solution culture. They found that the durum varieties had a greater reduction in plant weight and leaf growth rate with increasing salinity and also had higher concentrations of Cl^- in the leaves than bread wheat.

Three genotypes were identified by Munns *et al.* (2000) as having higher levels of Na exclusion than previously reported in durum wheat; however these genotypes were lacking important agricultural and adaptational characters such as early maturity, short straw and lodging resistance (Munns pers. comm.). Consequently, a backcrossing program to incorporate the Na exclusion trait into locally adapted genotypes was designed and undertaken for this thesis. A pre-requisite to this was a reliable screening technique and a knowledge of the mode of inheritance of the character.

A series of experiments were undertaken with the aim of finding efficient selection methods to identify suitable parental lines for subsequent crossing in the backcrossing program. Ideally, BC_1F_1 plants could be selected before anthesis, based on the Na concentration of the primary tiller. For this to be successful, the allele for exclusion must be fully or partially dominant. Backcrossing could then be carried out on later tillers of the heterozygous, low Na accumulating plants.

It is important to note that the work by Munns *et al.* (2003), suggesting that two genes control the Na exclusion character, was not published until after the experiments reported in this chapter were completed. Consequently the published two-gene hypothesis did not influence the design of these experiments.

5.2 Population construction

5.2.1 Introduction

The durum variety Kalka (released 2003) was chosen as the recurrent parent for the backcrossing program because it had boron tolerance introduced by backcrossing from a Chinese line, Lingzhi Baimong Baidamai, an important adaptational character in many areas where transient salinity is a problem. The crosses (Kalka#*Na49) and ((Wollaroi*Na49)*Kalka) had been made by Mr. Brenton Brooks, a PhD. student with the Waite wheat breeding unit. Kalka is derived largely from Wollaroi and related parentage; therefore both crosses are more or less equivalent to the first backcross to the recurrent parent (Kalka), and have been treated as such. All of the material described in this chapter is derived from these crosses. The procedure followed to produce the BC_3 lines from these crosses is complicated and it may be of assistance to refer to the flow chart in Appendix 1.

5.2.2 Materials and methods

BC_1F_1 derived F_2 field plots

The BC_1F_1 seeds were planted in the glasshouse and at maturity BC_1F_2 seed was harvested from forty-eight individual BC_1F_1 plants.

The F_2 seed of the forty-eight BC_1F_1 derived selections was sown in 4 row x 4.2 m plots in the standard method employed by the Waite durum breeding program (see Chapter 3). Tamaroi check plots were included every sixth plot (total of eleven). As variable quantities of seed of the BC_1F_1 derived lines were available, plant establishment ranged from a few plants per plot to 200 plants/m^2.

Tissue samples (15 whole tillers, or less where there were an insufficient number of plants) were collected from each plot in September 2000, dried, ground in a stainless steel mill and submitted for ICP analysis. Five BC_1F_1 derived lines with the lowest Na concentration were selected, with the aim of eliminating the homozygous high Na accumulating lines.

Reselected Na excluding BC_1F_3 plants from the selected BC_1F_1 lines (glasshouse)

Fifty F_3 seeds of each of the five BC_1F_1 derivatives were planted in ten 250mm plastic lined pots (5 seeds/pot) in the glasshouse (Chapter 4), together with five pots of both Kalka and Na49. At eight days, 150 ml of 0.5M NaCl solution was added to each pot. Seventy-eight plants, including at least a few selections from all five BC_1F_1 derivatives, were selected on the basis of plant type and at booting stage (Zadoks scale 45) the primary tiller of each was harvested separately, dried, ground and analysed using ICP spectrometry. The Kalka and Na49 plants were also analysed in the same way except that all five primary tillers from each pot were bulked so that there were five replicates of each of the two parental lines.

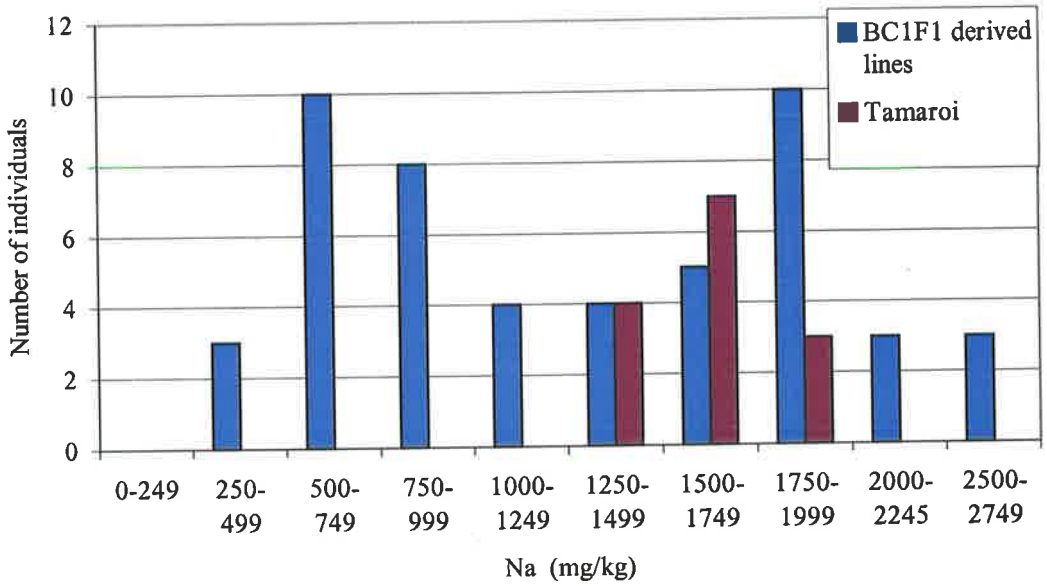
After sampling for ICP analysis, the plastic bags lining the pots were punctured and the pots watered liberally to flush the salt out to allow the plants to recover for seed production. Later tillers of the seven reselections with the lowest Na concentration were backcrossed to Kalka to produce BC_2F_1 seeds (Continuation of the population development is described in Section 5.3.2).

5.2.3 Results

BC_1F_1 derived F_2 field plots

The BC_1F_1 derived F_2 lines ranged in Na concentration from 450 to 2300mg/kg, with a mean of 1276±595. The Tamaroi check plots ranged from 1380 to 1890mg/kg with a mean of 1584±193 (Figure 5.1). The data were not subjected to statistical analyses because the lines were not replicated and the variation in plant density; however there were twenty-five BC_1F_1 derived lines with lower concentrations of Na than the lowest Tamaroi. The distribution of the BC_1 lines was bimodal with the low frequency occurring at approximately 1250 mg/kg (there are no individuals between 1180 and 1290). Separation at this point resulted in a ratio of 25:23 (low:high Na accumulation). The χ^2 value of 0.08 for two classes was not significant and therefore the distribution fits the 1:1 expected for single gene control of the Na exclusion trait, with the low Na accumulating lines being derived from a heterozygous individual.

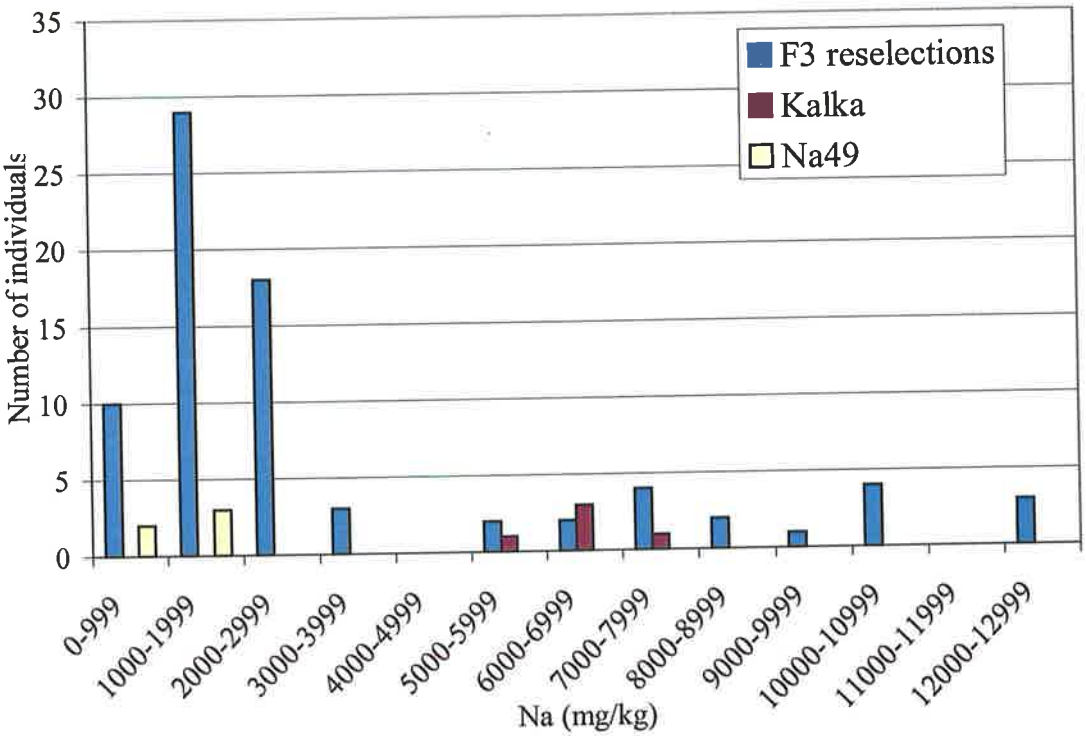
Figure 5.1. Frequency distribution of Na concentration in 15 whole tillers of 48 BC_1F_1 derived F_2 field plots relative to a commercial durum variety Tamaroi.



F_3 reselections of Na excluding plants from the BC_1F_1 derived F_2 lines (glasshouse)

Analysis of variance of the Na data from the parental lines Kalka and Na49 from the pot trial in the glasshouse indicated that they were significantly different ($P<0.001$), with mean Na concentrations of 6500±262 and 1450±80 mg/kg respectively. The F_3 reselections from the five low Na accumulating BC_1F_1 derived F_2 lines ranged in Na concentration of the primary tillers from 280 to 12800 mg/kg with a mean of 3475±3214 (Figure 5.2). A large peak in the frequency distribution of the reselections corresponded with the mean value for the Na excluding parent Na49, consistent with the recovery of a large number of progeny with the same level of expression of the Na excluding trait as Na49. At higher Na concentrations than that observed in Na49, the progeny are distributed around the mean of the recurrent parent Kalka, with an apparent minimum in the range 4000-4999mg/kg.

Figure 5.2. The distribution of 78 reselected F_3 single plants from five BC_1F_1 derived lines segregating for Na exclusion, relative to the donor parent Na49 and the recurrent parent Kalka. Plants grown in saline pots in a glasshouse.



The distributions of the individual F_3 reselections from each of the F_1 derived lines indicate that high and low Na types were recovered from all five families (Table 5.1), confirming that segregating plots had been correctly identified in the field.

Table 5.1. Classification of reselected F_3 single plants from F_1 derived BC_1 lines segregating for a Na exclusion character on the basis of Na concentration relative to the donor parent, Na49 and the recurrent parent, Kalka.

BC_1F_1 derived line	No. of reselections	Na classification	
		Low	High
(Kalka#*Na49)/1	12	8	4
(Kalka#*Na49)/2	20	18	4
(Kalka#*Na49)/5	22	14	8
(Kalka#*Na49)/9	13	11	2
(Wollaroi*Na49)*Kalka)/2	11	7	4
Total	78	58	20

5.2.4 Discussion

Under the hypothesis of a single dominant gene model of inheritance, the plots segregating for Na exclusion would be derived from heterozygous BC_1F_1 plants. In the F_3 reselections, the segregating lines would be expected to have a genotypic ratio of 3:2:3 (homozygous excluders:heterozygous:homozygous non-excluders) and with dominance this would equate to a phenotypic ratio of 5:3 (excluders:normal). With these assumptions, 49 Na excluding lines would be expected from the 78 tested. The χ^2 for the ratio of 58:20 observed has a value of 3.31, which is less than the 3.84 needed for significance at the 5% level of confidence.

With the data combined over the five BC_1F_1 lines, the two classes identified in the distribution were not significantly different from the expected ratio. Similarly, the frequency of Na excluders:non-excluders observed within the individual BC_1 F_1 derived

families was also not significantly different from expected, except in the (Kalka#*Na49)/2 family (Table 5.1). This family had a low:high ratio of 18:2 for Na classification, which has a χ^2 value of 4.84, greater than the 3.84 for significance at the 5% level. There are two possible reasons for the abnormal ratio. Firstly, by definition, 5% of families are expected to have ratios significantly different from that expected. Alternatively, this family may have had very low plant numbers in the F_2 field plot (<12) and proportionally more seed may have been harvested from the segregants excluding Na^+. Abnormal ratios are more likely to occur by chance in the lines with low F_2 plant numbers and this is likely to be exacerbated by selection pressure favouring Na excluding genotypes.

5.3 Development of selection techniques for detecting heterozygous Na excluding F_1 plants and development of a BC_3 derived population

5.3.1 Introduction

The most efficient way to utilize a new character is by backcrossing it into an otherwise adapted background. This is best achieved by identifying heterozygotes before anthesis to enable further backcrossing of selected plants in the same generation.

The experiment described here aimed to identify heterozygous F_1 plants grown in saline soil on the basis of ICP spectrometry analysis of the primary tiller, as well as use of this method to develop a BC_3 population segregating for the Na exclusion mechanism from Na49.

5.3.2 Materials and methods

Population development

Secondary tillers of seven F_3 reselections identified in the previous experiment (Section 5.2) as having the lowest Na concentration had been backcrossed (BC_2). Ten F_1 seeds from each cross were planted in the glasshouse and each plant backcrossed again (BC_3) without selection for Na uptake.

The BC_2F_1 plants were not screened for Na uptake because most plants were expected to be heterozygous for the trait on the basis of the argument below.

The F_3 reselections crossed to produce the BC_2F_1 plants would have segregated in a ratio of 3:2:3 (homozygous low Na : heterozygous : homozygous high Na). Consequently, of those selected for low Na accumulation, three fifths would have been expected to be homozygous. This means that three fifths of the BC_2 crosses would have been derived from parental crosses of the form NaNa x nana (Na representing the Na exclusion allele), while two fifths of the crosses would have been of the type Nana x nana. Hence, only one fifth of the BC_2F_1 seeds resulting from these crosses would not be expected to be heterozygous for the Na exclusion gene.

Development of screening method

Seed

Four F_1 seeds from each of the seventy BC_3 crosses and fifteen seeds of the parental lines, Kalka and Na49, were pre-germinated in petri dishes at 4°C for 7 days.

Soil

Three kilograms of damp potting soil (University of California mix) supplied by SARDI Plant Growth Services was weighed into 175mm diameter pots lined with plastic bags. The soil had been thoroughly mixed prior to weighing to achieve even moisture concentration. Although experimentally it would have been preferable to weigh out air dried soil, prior experience had shown that this potting mix is prone to become non-wetting with such treatment.

Experimental Design

The pots were divided into five blocks of ten. A single seed of Kalka and Na49 were planted at random in each block, along with two F_1 seeds from each of the seventy BC_3 crosses (total of 140 BC_3F_1 plants). Three pre-germinated seeds were sown in each pot, with observations made on single plants, not single pots. Eight days after seeding, 150mL of 0.5M NaCl solution was added to each pot. When the plants began to wilt, all pots were watered with 150mL of RO water.

At booting (Zadoks 43), the primary tiller was harvested and dried for 24 hours at 70°C. The samples were chopped using stainless steel scissors and analysed by ICP spectrometry.

Fourteen plants were not tested due to plant death or inadequate quantity of tissue for analysis.

After the tillers were harvested, the plastic bags lining the pots were punctured through each of the drainage holes of the pots. The pots were watered to excess to flush out salt from the soil and fertilised with approximately 4g of the complete fertiliser Nitrophoska ®. Unfortunately the recovery of plants in this experiment was not good, and many died without producing seed. Additional damage was done by mice during grain fill and before the seed was ripe. Consequently, seed was recovered from only twenty-one of the fifty-nine plants that were found to be carrying the Na exclusion allele and twenty-five plants of the sixty-seven without the allele.

5.3.3 Results and discussion

Analysis of variance on the two parents included in the experiment showed that a significant difference occurred between the two genotypes for Na concentration ($P<0.001$) (Table 5.2).

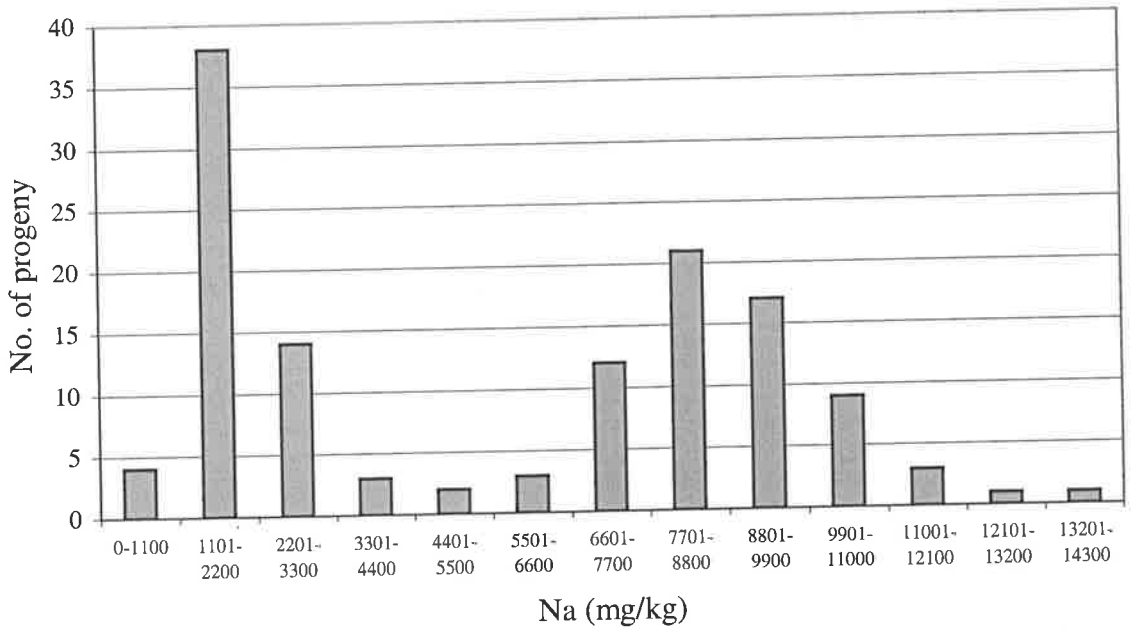
Table 5.2 Mean Na concentration (mg/kg at Zadocks 43) of five primary tillers of two parental genotypes grown in saline pots (10 dS/m ECe).

Kalka	Na49
9580	1186

L.S.D. ($P<0.05$) 2824

The Na concentration of the 126 BC_3F_1 plants tested ranged from 700-13,600 mg/kg, with a mean of 5555 mg/kg. As can be seen in Figure 5.3, two distinct peaks were clearly distinguishable which correspond with the parental means reported in Table 5.2.

Figure 5.3. Distribution of 126 BC_3F_1 progeny of the cross (Kalka#4*Na49) for Na concentration (mg/kg) in the primary tiller of plants grown in pots under glasshouse conditions.



If the separation between the two peaks is taken at 4400 mg/kg (no individuals between 3900 and 4600) the ratio of progeny in each category is 59:67 (low:high Na), or 46.8% carrying the Na exclusion allele. This is close to the 50% expected for single gene control of the Na exclusion character if all seven of the low Na BC_1F_3 plants selected for the final two backcrosses had been homozygous. The χ^2 value for the ratio observed is 1.14, which is less than the critical value of 3.84 ($P<0.05$). Therefore, the ratio is not significantly different from 1:1 ($P<0.05$).

It was expected that three fifths of the BC_1F_3 plants on which the BC_2 crosses were made would have been homozygous Na excluders and two fifths heterozygous, as described in the introduction to this section. If heterozygous plants had been selected for backcrossing it was expected that an excess of high Na (non-excluding) plants would be observed in this

experiment. This did not occur, suggesting that the seven plants selected for backcrossing were homozygous. Conformation of this conclusion is provided by the fact that the ratio of the two types of BC_3F_1 progeny derived from each of the seven BC_1 plants was not significantly different from 1:1, although with such small population sizes an accurate assessment of the segregation ratios was unlikely (Table 5.3).

Table 5.3. Classification of BC_3F_1 progeny derived from seven BC_1 plants on the basis of Na accumulation relative to the Na excluding donor parent Na49 and the non-excluding recurrent parent, Kalka.

BC_1 plant	Na excluding BC_3F_1 plants	Non-excluding BC_3F_1 plants
(Wol*Na49)*Kalka)/2/31	9	7
(Wol*Na49)*Kalka)/2/85	7	10
(Kalka#*Na49)/231	5	4
(Kalka#*Na49)/2/51	11	16
(Kalka#*Na49)/2/71	13	18
(Kalka#*Na49)/2/91	10	6
(Kalka#*Na49)/1/32	4	6

These results (Figure 5.3) confirm that selection of heterozygous F_1 plants for a backcrossing program is feasible for this gene.

5.4 Sodium uptake of the F_2 derived population in a pot experiment

5.4.1 Introduction

The data obtained in the previous experiment indicates that the Na exclusion trait is controlled be a single dominant gene. To validate this conclusion, a population of 196 single F_2 plants was examined from the survivors of the fifty-nine low Na F_1 plants reported in Section 5.3. A population of 36 F_2 selections was also selected from the high Na F_1 plants to check that these had been correctly classified in the previous experiment.

5.4.2 Materials and methods

Many of the BC_3F_1 plants did not recover from the salt treatment and mouse damage described in Section 5.3.2, so that the 196 plants included all of the F_2s harvested from the low Na F_1 plants. The F_2 seeds were grown in pots in the glasshouse to produce BC_3F_2 derived F_3 lines. These F_3 lines were concurrently tested for Na exclusion in the glasshouse and sown in multiplication rows in the bird proof enclosure in preparation for the field experiments described in Chapter 6. Three of the F_2 plants produced very little seed, all of which was planted in the multiplication row. Consequently only 193 of these lines were tested for Na uptake

Seed

Twelve seeds from each of the 229 F_2 derived F_3 lines (193 derived from low Na F_1 plants and thirty-six from homozygous high Na F_1 plants) were pre-germinated in petri dishes at 4°C for seven days, along with sixty seeds of the parental lines, Kalka and Na49. Each genotype was grown in a single pot, except for the parental lines, which were replicated

four times; hence there were 237 pots in total. Seven pre-germinated seeds were planted in each pot, which were thinned to six after eight days to enhance seedling uniformity.

Soil

The pots and soil were prepared by the same methods as are described in Section 5.3.2 of this thesis.

Experimental Design

The pots were arranged into four blocks on glasshouse benches (3 blocks of 59 pots and 1 block of 60). The F_2 derived lines were allocated randomly to a single pot each throughout the four blocks, while the parental lines were included once within each of the four blocks.

Salt was added to each pot after eight days by the addition of 150mL of 0.5M NaCl. The salinity of the soil after addition of salt was 0.89dS/m ($EC_{1:5}$), which is approximately equivalent to 8.9dS/m (ECe). At watering each pot received 150mL of water. Watering was performed whenever plants began to wilt, thereby avoiding waterlogging but maximising Na uptake.

Whole plants were harvested at anthesis (Zadocks 60), washed in RO water, oven dried at 70°C and chopped finely using stainless steel scissors, before being submitted for analysis by ICP spectrometry.

5.4.3 Results

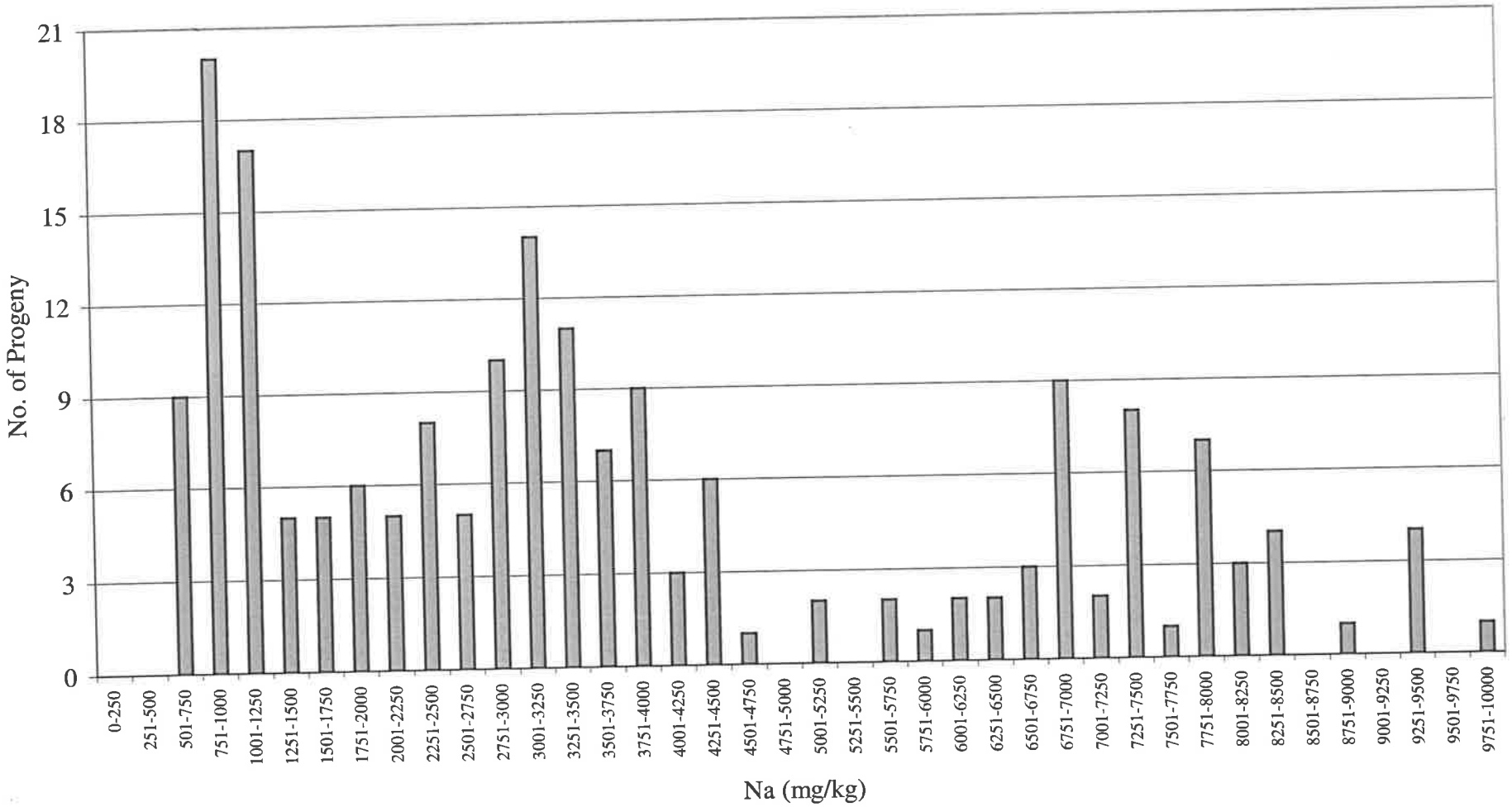
Analysis of variance for the Na data of the two parents indicated that they were significantly different ($P<0.001$). The mean Na concentration of the whole tops (dry) of

Kalka was 7050±38 mg/kg, while Na49 contained 985±91 mg/kg. There was no significant effect associated with replicate, allowing a confident comparison of the F_2 derived lines across all blocks.

The Na concentration of the 193 F_2 derived F_3 lines that were selected from low Na BC_3F_1 plants varied from 650 to 9800mg/kg. As can be seen in Figure 5.4, three distinct peaks occur in the distribution of the F_2 derived progeny. Two of these correspond closely with the parental means, while the third peak is intermediate to the Na concentration of the two parents.

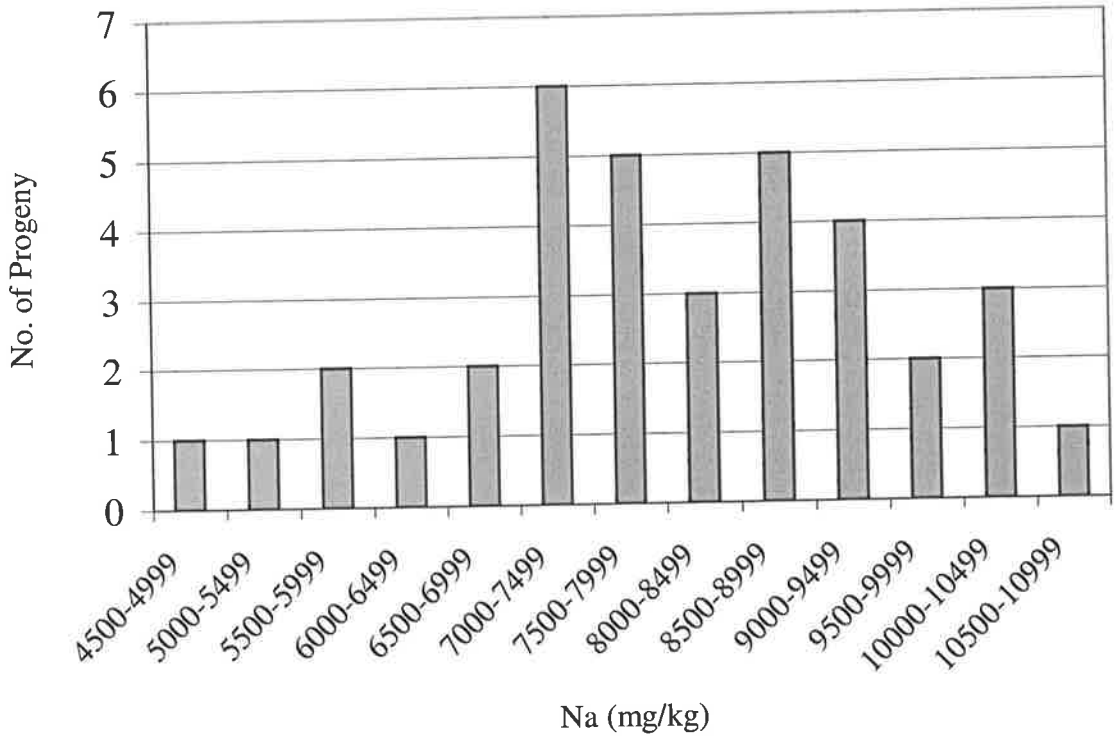
If separation of the three peaks of the tri-modal distribution is made at 1250 mg/kg and 5500 mg/kg, the ratio of progeny is 46:97:50, which is very close to the 48:97:48 expected for F_2 derived progeny for a single dominant gene. The χ^2 value of 0.16 is less than the critical value of 5.99 ($P<0.05$) and is therefore not significantly different from the 1:2:1 ratio expected. The intermediate peak represents heterogeneous F_3 lines derived from heterozygous F_2 plants.

Figure 5.4. Frequency distribution of Na concentration in the whole shoot of 193 F_2 derived F_3 lines selected from Na excluding BC_1F_1 plants of the cross (Kalka#4*Na49). Plants were grown in saline pots in a glasshouse.



The Na concentration of the thirty-six F_2 derived F_3 progeny selected from high Na accumulating F_1 plants ranged from 4800 to 10600 mg/kg with a mean of 8040 ±1453 mg/kg (Figure 5.5). This is comparable with that of the high Na lines segregating from the Na excluding BC_3F_1s. This group appeared to be normally distributed with a mean of 7477 ±1042 mg/kg (Figure 5.4).

Figure 5.5. Distribution of 36 F_2 derived F_3 lines selected from high Na accumulating F_1 plants of the cross (Kalka#4*Na49).



These data indicate that the high Na accumulating F_1 plants were correctly identified and that they were homozygous non-excluders.

5.5 Progeny testing of BC_3F_2 derived lines for Na exclusion in the field

5.5.1 Introduction

The F_2 derived F_3 lines grown in the bird proof enclosure were harvested to provide F_2 derived F_4 seed. Only 186 out of the 196 BC_3F_2 lines produced enough seed to be planted in field trials. The amount of seed available determined the number of sites at which each line could be tested. All 186 were sown at Two Wells, 164 were sown at both Two Wells and Port Pirie, and 124 had enough seed to be sown at Redhill as well as the other two sites. These field sites were located primarily to investigate the effect of the Na exclusion trait on tolerance to transient salinity, as measured by grain yield and grain size (screenings) (see Chapter 6.).

The recurrent parent Kalka was included as the check variety. A Na excluding BC_1F_3 derived line (Kalka#*Na49)/2/85 was included to represent the excluding parent rather than Na49 itself. This line was derived from one of the BC_1F_3 derived Na excluding plants identified in the glasshouse experiment described in Section 5.2 and was one of the seven donor parents for the BC_2 crosses described in Section 5.3.1. It was the only one of the seven lines to have similar maturity, height, boron tolerance and growth habit to Kalka. A preliminary pot experiment was conducted to confirm that the level of Na exclusion of (Kalka#*Na49)/2/85 was the same as that of the donor parent Na49.

The field experiments were sampled for Na concentration to progeny test the lines classified previously in the glasshouse experiment (see 5.4), with the aim of verifying the single dominant gene theory. To obtain these data all plots were sampled at Port Pirie. The lines not sown at Port Pirie were sampled at Two Wells, along with check plots.

5.5.2 Materials and methods

*Preliminary experiment to validate the adoption of the adapted line (Kalka#*Na49)/2/85 as an alternative to Na49 as a Na excluding check.*

Six replicates of Kalka, Na49 and the BC_1F_3 derived line (Kalka#*Na49)/2/85 were sown in six completely randomised blocks. Each genotype was represented in each block by a single pot containing six pre-germinated seeds. These were thinned to 5 after seedling emergence. The preparation of pots, salt treatment, tissue sampling and ICP spectrometry analysis were the same as that described in 5.4.

Field experiments

The field sites were located on farms known to be affected by transient salinity. The choice of farm was based primarily on discussion with agronomists and farmers in areas that were known to have high levels of subsoil salinity from the map prepared by PIRSA Land Information (2001) (Figure 2.1). At each site, an apparently uniform area, large enough to accommodate the experiment was selected using an EM38 conductivity meter in the upright orientation, measuring the average conductivity of the soil to a depth of 1.5m. The average ECe to this depth was 13, 22 and 18ds/m for the Redhill, Port Pirie and the Two Wells sites respectively.

The field plots were sown in the usual 4 row x 4.2m plots used by the Waite durum wheat breeding program as described in Chapter 3. At all sites the recurrent parent Kalka was included as the check variety every sixth plot throughout the experiment.

The Two Wells experiment consisted of two replicates of 266 entries. Each replicate consisted of 127 BC_3 F_2 derived progeny entered once and fifty-nine entered twice. The other twenty-one entries were the Na excluding standard (Kalka#*Na49)/2/85. A grid of Kalka check plots was also included to provide a measure of the spatial variation across the experiment. This consisted of 113 plots, occurring in every sixth plot, starting in column one in odd numbered bays and column four in even numbered bays. The total number of plots was 645 arranged into fifteen bays and forty-three columns.

The Port Pirie experiment consisted of two replicates of 172 entries, 164 BC_3F_2 derived progeny and eight entries of the Na excluding check (Kalka#*Na49)/2/85. A grid of seventy-five check plots (Kalka) was also included every sixth plot as described for the Two Wells experiment. The total number of plots was 419 and one extra plot added to fit the experiment layout of fifteen bays and twenty-eight columns.

One hundred and twenty-four BC_3 F_2 derived progeny and eleven entries of (Kalka#*Na49)/2/85 were included in the Redhill experiment. All entries were replicated twice. A grid of sixty check plots (Kalka) was also included every sixth plot as described for the Two Wells experiment. The total number of plots was 330 arranged into fifteen bays and twenty-two columns.

The Port Pirie and Redhill experiments were sown on the eleventh of June, followed by the Two Wells experiment on the twenty-fifth of June 2003. These seeding dates were later than optimal as a result of the quarantine restrictions placed on the Waite Campus in response to the discovery of the disease Wheat Streak Mosaic Virus. The Two Wells site was sown particularly late because the Waite Durum Breeding Unit had sown their

experiments at that site on the second day after the restrictions were lifted and before the backcross derived lines could be harvested from the bird proof enclosure. Consequently, the experiment described in this study was sown only after the rest of the durum breeding experiments had been sown.

The Port Pirie experiment was the first to reach anthesis (judged to be when the Kalka check plots reached anthesis) so was sampled for Na concentration at that time on the tenth of September 2003. Twelve whole tillers were sampled at random from each plot and placed in a paper bag. The samples were dried at 80°C for thirty hours before being ground in a stainless steel mill and sieved through a 2mm screen. Each sample was then digested in nitric acid and analysed for elemental composition using ICP spectrometry.

The Two Wells experiment included some BC_3F_2 derived progeny not represented at the Port Pirie site and these were sampled on the first of October 2003. Two Kalka check plots and a single (Kalka#*Na49)/2/85 plot were sampled from each of the fifteen bays to provide a measure of spatial variation and Na data of the parental lines for comparison with the backcross derived lines. The samples were collected, dried, milled and analysed as described for the Port Pirie samples.

5.5.3 Results

*Preliminary experiment to validate the adoption of the adapted line (Kalka#*Na49)/2/85 as an alternative to Na49 as a Na excluding check.*

The analysis of variance on the Na data from the pot test confirmed that the F_1 derived BC_1 line (Kalka#*Na49)/2/85 was not significantly different in Na concentration from the

donor parent, Na49. It also confirmed that both of these lines accumulate significantly less Na than the recurrent parent Kalka (Table 5.4.). There was no significant difference between replicates.

Table 5.4. Na concentration (mg/kg) in whole plant tops of three genotypes grown in saline pots (10 dS/m ECe).

Genotype	Na (mg/kg)
Na49	880
Kalka	7822
(Na49*Kalka)/2/85	937

L.S.D. ($P<0.05$) = 702

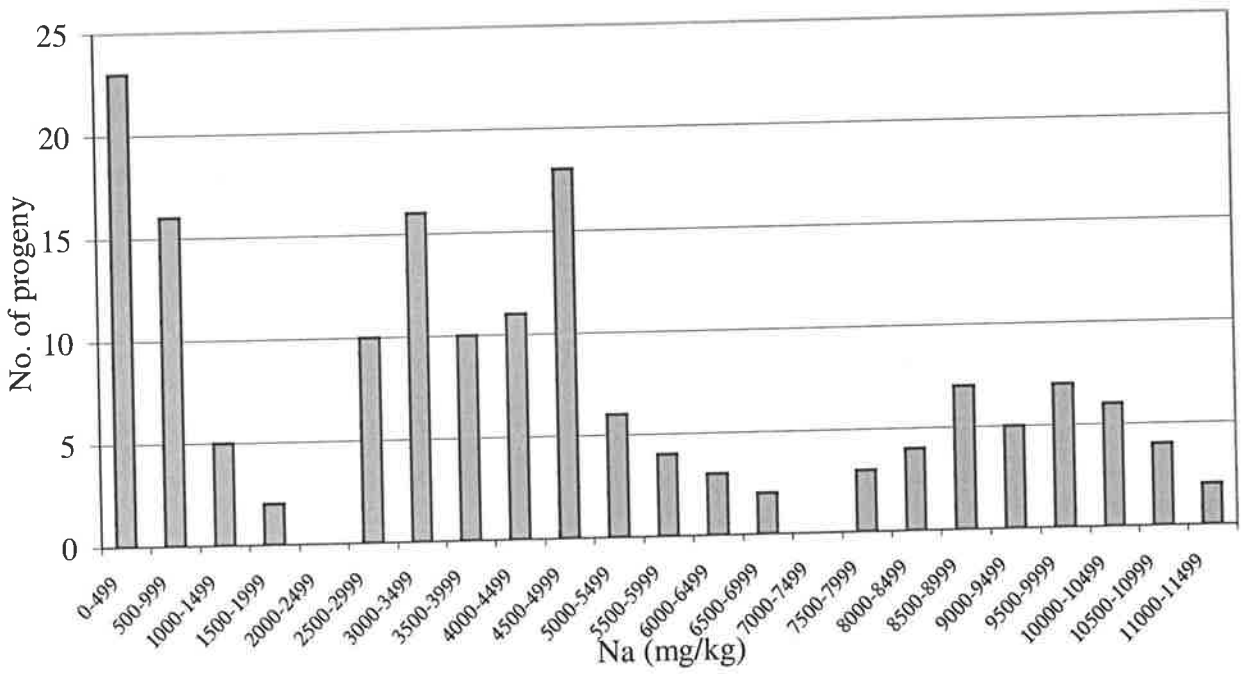
This validated the use of (Kalka#*Na49)/2/85 as the Na excluding check genotype as an alternative to Na49 in the field experiments.

Field experiments

Analysis of variance of the Na concentrations obtained for the BC_3F_2 derived F_4 lines grown at Port Pirie indicated that genotype was highly significant ($P<0.001$) and that replicate had no significant effect.

As can be seen in Figure 5.6 the progeny fall into three clearly defined categories, similar to the results obtained from the glasshouse test of the F_2 derived F_3 lines (see Figure 5.4).

Figure 5.6. The frequency distribution of Na concentration in whole tillers of 164 BC_3F_2 derived F_4 lines selected from low Na F_1 plants of the cross (Kalka#4*Na49). Data obtained from plots sampled at Port Pirie.



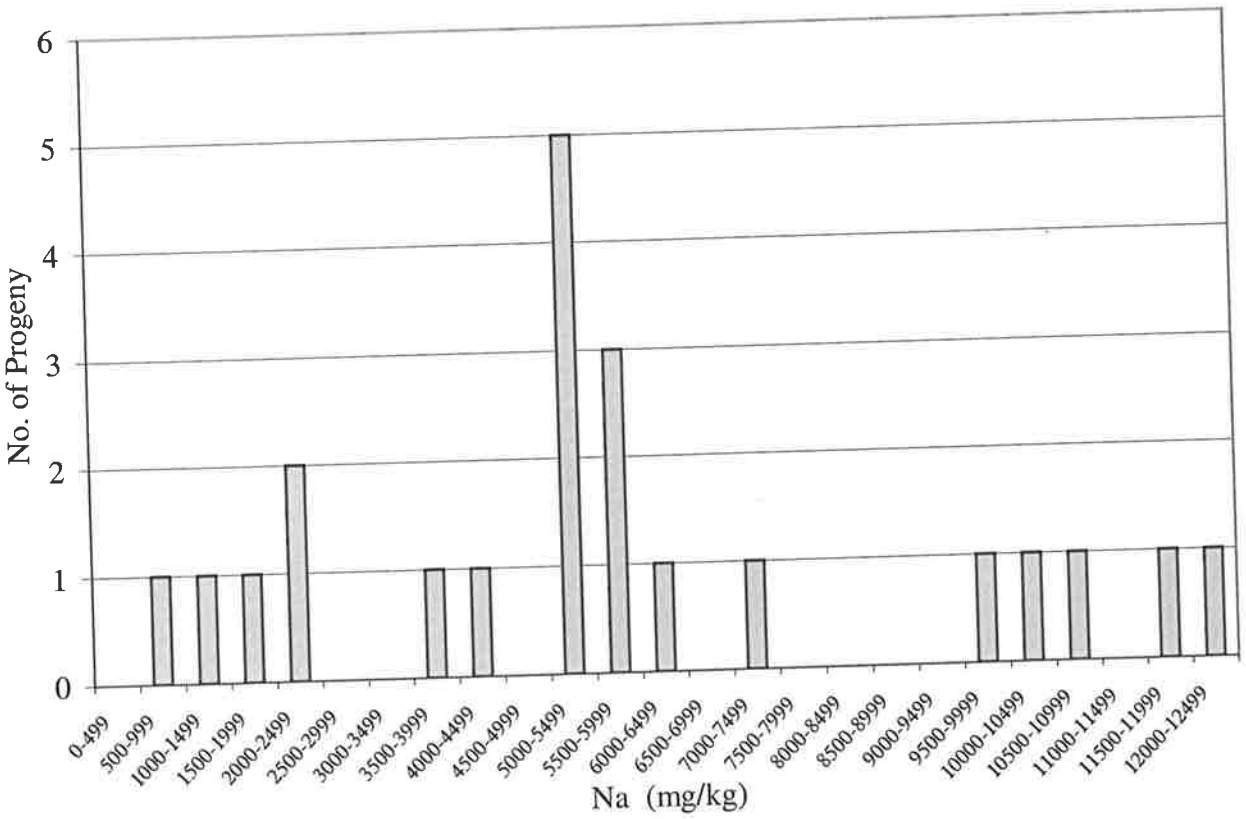
The mean Na concentration of the seventy-five Kalka check plots was 9373±154 mg/kg, while the mean Na concentration of the sixteen (Kalka#*Na49)/2/85 plots was 856±87 mg/kg. It is clear that the highest and lowest peaks in the distribution (Figure 5.6) are consistent with the parental means. If 2500 and 7000mg/kg are taken as the points of separation between the three classes, the ratio of progeny is 46:80:38, which is very close to the 41:82:41 expected for F_2 progeny segregating for a single gene. The χ^2 value of 0.88 obtained is well below the 5.99 needed for the observed to be significantly different from expected ($P<0.05$). This confirms the result obtained in the glasshouse experiment (Section 5.4.3).

The lines not included in the Port Pirie experiment were sampled at Two Wells. Analysis of variance of the Na concentration of these BC_3F_2 also indicated that genotype was highly

significant ($P<0.001$) and that replicate had no significant effect. Consequently, the Na concentration data obtained from both sites was not spatially analysed.

The frequency distribution of the F_2 derived progeny from the Two Wells site for Na concentration (Figure 5.7.) shows that the progeny cluster in three groups similar to the results observed at Port Pirie (Figure 5.6.).

Figure 5.7. The frequency distribution of Na concentration in whole tillers of 22 BC_3F_2 derived F_4 lines selected from low Na F_1 plants of the cross (Kalka#4*Na49). Data obtained from plots sampled at Two Wells.



The mean Na concentration of the thirty Kalka check plots was 10317±420 mg/kg, while the mean Na concentration of the fifteen (Kalka#*Na49)/2/85 plots was 930±52 mg/kg. If the three clusters of progeny are separated at 3000 and 8000 mg/kg, the parental means of

930mg/kg and 10317mg/kg are close to the mean of the lowest and uppermost clusters respectively. If these clusters are taken to be equivalent to the three peaks in Figure 5.6, they add 5, 12 and 5 to the three categories of F_2 derived progeny. Consequently the ratio becomes 51:92:43 and this has a χ^2 value of 0.17 when compared to the expected ratio of 46.5:93:46.5 for single gene control of the trait. This χ^2 is less than the critical value of 5.99 ($P<0.05$) and is therefore not significantly different from the 1:2:1 ratio expected.

The comparison of the Na characterisation of the F_2 derived lines in the F_3 and F_4 experiments provides an indication of the repeatability of the screening for the Na exclusion trait. Table 5.5 summarises the frequency with which lines were characterised into each Na category in each test (Note: not all lines were in both experiments)

Table 5.5. The distribution of progeny from the cross (Kalka#4*Na49) within three categories of Na uptake. The lines were tested as F_2 derived F_3 lines and retested in the F_4.

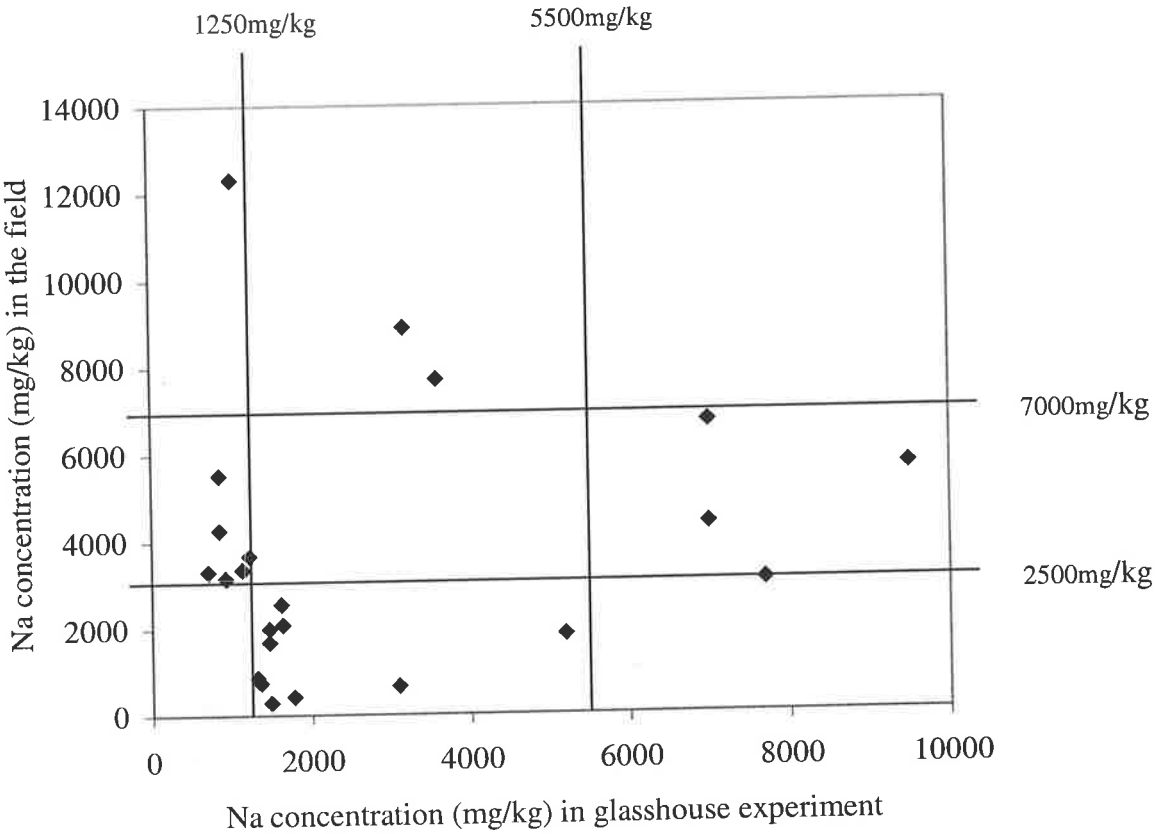
		F_4 Retest (Pt. Pirie and Two Wells)		
		Low	Segregating	High
F_3 Test	Low	39	6	1
(glasshouse)	Segregating	10	81	2
	High	0	4	40

Of the 183 progeny included in both experiments (three not included in glasshouse experiment), 160 were included in the same Na category in both tests. Twenty-three were misclassified, of which, only one was classified as a low sodium line in one experiment and high sodium in the other. This misclassification probably resulted from an

experimental error, such as the use of incorrect seed in either the pot test or the field experiment.

Of the remaining twenty-two misclassifications, sixteen occured between the segregating and low classes, reflecting the overlap between the homozygous excluders and the segregating class in Figure 5.4. Inspection of the distribution of the F_2 derived F_3 lines in the glasshouse experiment (Figure 5.4.) reveals that the tri-modal distribution is disjunct between the segregating class and the high Na class, but continuous between the Na excluding and segregating classes; therefore it is not unexpected that misclassification will occur at the lower point of separation. When the Na concentration in the glasshouse and the field is compared for the misclassified lines (Figure 5.8), all of the segregating lines misclassified in the glasshouse as low Na accumulators lie close to the point of discrimination (1250 mg/kg), while this is also true for eight of the ten low Na lines misclassified as segregating. This confirms that misclassification has occurred primarily because of the ambiguity of the classes (Figure 5.4) and that most of them are close to the class in which they actually belong on the basis of the more critical field experiment.

Figure 5.8. Comparison of the Na concentration (mg/kg) in the F_3 glasshouse experiment and F_4 field plots for the misclassified F_2BC_4 derived lines of the cross (Kalka#4*Na49).



5.5.4. Discussion

The distribution of the F_2 derived progeny in the glasshouse experiment suggested strongly that the character was controlled by a single dominant gene of large effect (Figure 5.5). The distribution was clearly tri-modal and had a χ^2 not significantly different from the expected 1:2:1 ratio. The mean of the lowest peak was consistent with the mean of the Na excluding donor parent Na49 indicating that no gene/s contributing to Na exclusion had been lost during backcrossing. The mean of the high Na peak was consistent with the high Na recurrent parent Kalka. No evidence of transgressive segregation was observed.

The progeny test carried out on the F_4 field plots validated the single dominant gene theory. The distribution of progeny into three classes with an approximate ratio of 1:2:1 was even more clearly defined by the disjunct distribution of these classes.

The results of these experiments establish that the glasshouse method is a sufficiently accurate method to characterise durum lines for Na exclusion on the basis of analysis of the vegetative parts of the plant. Of the 183 lines included in both the glasshouse test and the field experiments, only twenty-three were misclassified and only one line was misclassified between high and low Na classes. A high proportion of the misclassifications (sixteen out of twenty-three) occurred between the low Na and segregating categories due to the overlap of the homozygous low Na and heterozygous classes in the frequency distribution from the glasshouse screening. If greater accuracy is required, it is likely that the best way to improve discrimination would be to use more replication and increase the sample size.

The small sample size (six tillers) in the glasshouse experiment may explain why many of the ten lines that were originally classified as being high or low Na, were reclassified as segregating in the field experiment. As the Na exclusion trait is controlled by a single dominant gene, the six plants representing a heterogeneous segregating line may, by chance, have had a relatively high number of either high or low Na types. Consequently, the mean Na concentration of such a segregating line could have had a value which would fall within the range of the homozygous genotypes. The field data obtained for each line represented the mean Na concentration of twenty-four plants (twelve tillers x 2 replicates). Clearly, the chance of a population of six plants having a predominance of one homozygous type is much higher than with a population of twenty-four.

5.6 Discussion of Chapter 5

Improving the salt tolerance of durum wheat is likely to increase the area of the southern Australian cropping zone in which durum wheat can reliably be produced. The mapping work led by Maschmedt (PIRSA Land Information, 2001) (Figure 2.1) shows that approximately 60% of the cropping zone of South Australia has subsoil salinity levels in excess of 4 dS/m ECe, which is considered to be the level at which salinity begins to effect crop yield (Maas and Hoffmann 1977).

Reducing the amount of Na accumulating in the leaves of durum wheat is expected to greatly improve its salt tolerance. To achieve this, a genetic source of Na exclusion is required, along with an understanding of its inheritance and a screening method that accurately identifies the progeny in a breeding program that are carrying the allele(s). If the principal method of introgressing the character is backcrossing, a screening method that is able to separate, pre-anthesis, heterozygous F_1 progeny carrying the allele(s) conferring the trait from homozygous normal plants will shorten the time between successive rounds of crossing. In the work described here, all these objectives have been achieved.

The tetraploid landrace, Na49, identified by Munns *et al.* (2000) has a significantly lower Na accumulation than current commercial durum varieties and a similar Na concentration to the hexaploid wheats currently grown in the areas affected by subsoil salinity levels above 4dS/m. Apart from the Na excluding ability of this line, it is an unattractive parent for producing improved varieties for South Australia, having very late maturity and tall, weak straw and it is likely that it carries many less obvious deleterious characters affecting adaptation, disease resistance and grain quality. Several cycles of backcrossing are likely to be the most suitable option for breeders to introgress the Na exclusion character into

commercially viable material with the number of cycles being determined largely by the genetic distance between the two parents and linkage drag of undesirable alleles associated with the Na exclusion locus. It is now clear from the field trials that the effect of linkage drag is significant, as in the absence of a salt stress, the lines carrying the Na exclusion trait are lower yielding than the high Na types (Chapter 7). The highest yielding Na excluding lines in these trials should be used as donor parents in future backcrossing programs.

Many breeders have used backcrossing effectively as the most efficient way to transfer new traits controlled by major genes into otherwise adapted backgrounds. For example, the breeding of the hexaploid wheat variety BT-Schomburgk by transferring the boron tolerance gene *Bo1* from Halberd into the background of Schomburgk (Rathjen *et al.* 1995).

The inheritance of the Na exclusion in the (Na49*Kalka) cross is controlled by a single dominant gene or chromosomal region, which enables the selection of heterozygous F_1 plants in a backcrossing program. As can be seen in Figure 5.3, full recovery of the Na exclusion character is seen in the BC_3F_1 heterozygous progeny. When single F_2 plants are selected from the heterozygous BC_3F_1 plants, they segregate in the 1:2:1 (homozygous excluders : heterozygous : homozygous non-excluders) ratio expected for single gene control of the trait (Figures 5.4, 5.6 and 5.7). In the population derived from three backcrosses (Kalka#4*Na49), where the percentage of Na49 background is greatly reduced, full recovery of the Na exclusion trait was achieved without any evidence of more than one gene controlling Na concentration. In contrast, Munns *et al.* (2003), with a different locally adapted parent, found that the trait was controlled by two dominant genes and that one of these was located on the long arm of chromosome 2A. An investigation

into these conflicting conclusions about the genetic control of the Na exclusion trait is described in chapter 6.

The plastic lined pot screening method described in Chapter 4. was developed as a reliable and inexpensive method of screening plants under uniform saline conditions. It can be set up in any environment that is suitable for plant growth without the use of specialised and/or expensive infrastructure and laborious technical requirements. The only requirements are that drips (from a leaking glasshouse roof for example) are not allowed to reach the pots, and that light intensity and air flow are relatively even across the experiment. If these factors are taken into consideration, the water usage of the plants is sufficiently uniform so that the salt concentration in the soil solution remained similar for all pots for the duration of the experiment. The only on-going technical requirement, for an experiment assessed at anthesis, was to water all pots with the same volume when the plants begin to wilt.

This method has proved to be a reliable and repeatable way of assessing plants for Na exclusion. The frequency of misclassification of lines was shown in two separate experiments to be reasonably low. The 36 F_2 lines selected randomly from the high Na BC_3F_1 plants (Figure 5.4) all had Na concentrations in the primary tiller equivalent to the recurrent parent Kalka, indicating that none of the high Na BC_3F_1 plants had been misclassified in the previous generation. The 193 F_2 selections taken from the BC_3F_1 plants classified as being heterozygous low Na plants represented twenty-two BC_3F_1 derived families, all of which segregated for both high and low Na F_2 derived lines.

The retesting of 183 of the 193 F_2 derived lines in the field experiments identified only twenty-three misclassifications of which most had occurred between the low Na and segregating classes. This revealed that the glasshouse method is less definitive for plants accumulating lower Na concentrations. If greater accuracy is required it is probable that the use of more replication would be of benefit.

It is expected that reducing the Na uptake of durum wheat will have a positive effect on the salt tolerance, and consequently adaptation of durum to southern Australian soils. A source of Na exclusion has been identified which appears to be simply inherited and suitable for introgression into locally adapted material. All of the important requirements are in place to make rapid industry progress through breeding.

Chapter 6.

AN INVESTIGATION INTO THE CONFLICTING CONCLUSIONS MADE ABOUT THE GENETIC CONTROL OF THE Na EXCLUSION OF Na49

6.1 Introduction.

An independent study of the genetic control of the Na exclusion character conferred by Na49 has been reported by Munns *et al* (2003). They concluded that the trait was controlled by duplicate dominance epistasis from two loci. This is a very different conclusion to that reached in Chapter 5. In an attempt to clarify the situation a further two experiments were carried out.

The results obtained by Munns and her co-workers (2003) were from an F_2 derived population from a cross between Na49 and Tamaroi. This population could have been expected to segregate for the same single gene identified in Chapter 5 unless another Na exclusion gene or genes was present in Kalka, but not in Tamaroi.

The pedigree of Tamaroi is (RH860193*Altar84). The breeders line RH860193 has the pedigree ((Guillemot*Kamilaroi)*((Wells*56111)*Guillemot), while the two parents of Kamilaroi, (Durati*Leeds), both resulted from crosses with Wells. Consequently, Tamaroi can be considered to be primarily a cross between the CIMMYT variety Altar84 and a parent that is largely derived from Wells and Guillemot.

The pedigree of Kalka is ((Wollaroi*((Linghzi Baimong Badamai*Yallaroi#)*RH880009)). The breeders line RH880009 has the pedigree (Yallaroi*(Tam1B-17*Kamilaroi)), where Tam1B-17 was a synonym of 56111 and was an

F_2 selection from an unknown CIMMYT cross. The pedigrees of Yallaroi and Wollaroi are (Kamilaroi*Guillemot) and ((Tam1B-17*Kamilaroi)*(Rokel*Kamilaroi)) respectively, which again are largely derived from Wells and Guillemot. The Chinese landrace Linghzi Baimong Baidamai is very different to the other parents genetically, but only contributes a small portion of the parentage of Kalka (6.25%).

The main difference between the pedigrees of the two varieties is Altar84 in the parentage of Tamaroi and consequently it is the parent that is most likely to be responsible for differences between the two varieties. While the Na accumulation/exclusion of Altar84 has not been investigated, Munns (pers. comm.) does not believe that there is any substantial variation within commercial durum varieties and almost certainly not of the magnitude of the effect in Na49. Munns has observed slightly lower Na accumulation in the variety Wollaroi, but this was inconsequential compared with the level of exclusion of Na49. Locally, it has been observed that Altar 84 and some of its derivatives have been much more vigorous than other lines at some locations (Rathjen pers. comm.), but at present this is thought to reflect tolerance to Zn deficiency.

Some comparisons of the Na accumulation of Tamaroi and Kalka have been made which indicate that the Na uptake of the two varieties is comparable. Lui (unpublished data) sampled field trials at Two Wells in 1998 and measured the Na concentration in whole tillers. The Na concentrations were 8580 and 9858 mg/kg in Tamaroi and Kalka respectively. Also, a single pot of Tamaroi was included with the experiment described in section 5.2. The Na concentration of the Tamaroi sampled from this pot was 6800mg/kg, compared with 6500mg/kg in Kalka (mean of five plants). The LSD (5% level) was 550, so this non-replicated Tamaroi observation was not significantly different from Kalka.

Munns *et al.* (2003) based their study on a population of one hundred F_2 derived lines from the cross (Na49*Tamaroi) and in contrast to the work reported in this thesis, they recovered a very low proportion of high Na accumulating (defined by being comparable with Tamaroi) F_2 derived progeny. The Na excluding parent Na49 was an unadapted landrace, derived with very little selection for traits of importance in commercial production in Australia. It has late maturity and very tall weak straw. Conversely, Tamaroi was developed for high input commercial production in the Australian environment. Consequently, the genotypic differences, or polymorphisms between Tamaroi and Na49 will be very great. The variation in progeny derived from the wide cross between these parents can be expected to be considerable and range from morphological to metabolic.

The wide cross between Tamaroi and Na49 may also have resulted in an abnormal segregation ratio due to unknown genetic incompatibilities. Distorted segregation ratios have previously been reported in many wide crosses. For instance, *Lablab purpureus* (Konduri *et al.,* 2000), mungbean (*Vigna radiata*) (Lambrides *et al.,* 2000) and hexaploid wheat (*Triticum aestivum*) (Kammholz *et al.*, 2001). If segregation distortion was the reason for the low proportion of Tamaroi types, it is hypothesised that in a backcross derived population, the genetic factors affecting segregation would have been eliminated.

The population developed for this project was derived from the third backcross to Kalka, a locally adapted variety. After four cycles of crossing, the proportion of chromatin from the donor parent will be approximately one sixteenth. This would have substantially reduced the influence of extraneous variation from Na49 for other characteristics such as maturity and growth rate, which could indirectly influence Na exclusion.

The plant material analysed for Na concentration by Munns *et al.* (2003) was also quite different to that described in this thesis, as they sampled the blade of leaf three, ten days after emergence from the sheath of the second leaf. Apart from the lower concentration of Na in the blade, they also observed that Na49 had a higher sheath to blade ratio for Na, through an ability to sequester Na from the xylem into the leaf sheath. It was argued that this latter trait was controlled by the QTL on chromosome 2A and that a second locus (chromosomal location unidentified) controlled whole plant exclusion (Munns pers. comm.).

The selection method implemented in the backcrossing program undertaken at the Waite Institute was quite different to that published by Munns *et al.* (2003). Selection of low Na genotypes was based on the Na concentration of whole tillers or whole plants at anthesis, rather than on the concentration in leaf blades. Consequently, plants carrying the sheath retention gene would have only been selected by chance. The probability of a single gene being present purely by chance can be calculated by the formula $P=(^1/_2)^n$, where n is the number of crosses without selection. Hence, in the (Kalka#4*Na49) population, only 6.25% of fixed lines selected from the last cycle of backcrossing could be expected to carry the sheath retention gene and the linked marker on chromosome 2A. This percentage is based on the assumption that these segregate independently of the gene controlling whole plant exclusion.

Another possibility arises from the difference in the age of the plant when sampled as the level of expression of the two genes may vary between the seedling and adult plant stages. At the seedling stage the two traits (root exclusion and sheath retention) may have an equal

effect on the Na concentration, but as the plant gets older and Na accumulates over time, the actual quantity entering the plant through the roots may become the dominant factor.

One final hypothesis for the low frequency of high concentration parental types recovered in the F_2 derived population of Munns *et al.* (2003), was that one or more of the F_1 plants grown to produce the F_2 seed was actually a selfed Na49 plant due to an error in crossing.

The hypotheses that potentially explain the differing conclusions about the genetic control of the Na exclusion trait, can be summarised as follows:

1) The locally adapted parents (Tamaroi and Kalka) of the two populations differ in Na exclusion, as one of the two loci segregating in the (Na49*Tamaroi) population, was already present in Kalka.

2) The genetic distance between the two parents of the population of Munns *et al.* (2003) results in extreme phenotypic variation in characters such as maturity, vigour and growth rate, which indirectly affected Na accumulation.

3) Genetic incompatibilities between Na49 and Tamaroi were responsible for segregation distortion in the F_2 derived progeny of the wide cross.

4) Sampling the whole tiller, or whole plant at anthesis has only allowed selection for the root exclusion trait in the population developed at the Waite. If the second gene (controlling sheath partitioning) segregated independently of root exclusion, it could have

been lost by chance during the backcrossing as only 6.25% of BC_3 derived lines would be expected to carry the allele.

5) A difference in the level of gene expression occured between seedlings and adult plants, so that segregation for two genes would be observed in the Na concentration in seedlings, while only one had a significant effect on the Na concentration in adult plants.

6) One or more of the (Na49*Tamaroi) F_1 plants was a selfed Na49 seed, due to an unsuccessful emasculation during crossing. All "F_2" seeds selected from this plant would be Na excluding parental types, distorting the segregation ratio.

To clarify the situation, the (Tamaroi*Na49) population from the CSIRO was tested at the Waite Institute under the conditions described in this thesis, while thirty lines from the (Kalka#4*Na49) population were tested by Munns at the CSIRO Division of Plant Industry under their conditions. The thirty (Kalka#4*Na49) lines were also probed for the presence of the marker on chromosome 2A, as it was argued that if the sheath retention trait had been lost during the backcrossing process, the marker would not be present.

6.2 Materials and methods

*Na uptake of the (Tamaroi*Na49) population at anthesis*

Ten seeds of each of the ninety-nine F_2 derived F_3 lines forwarded (selection 48 was missing) by Munns were pre-germinated on filter paper in Petri dishes at 4°C for seven days, along with forty seeds each of Na49 and Tamaroi. One hundred and seven pots were prepared in the same way as those described in Chapter 5, section 5.2.2. These were placed

on glasshouse benches in three blocks of twenty-seven and one of twenty-six. The ninety-nine lines were randomly allocated to a single pot each, while single pots of Tamaroi and Na49 were included in each block to enable an analysis of variance to be performed. Six pre-germinated seeds were planted in each pot, which were later thinned to five by removing the least vigorous plant where six had emerged.

When the Tamaroi plants reached anthesis the whole tops were harvested from all pots, dried, ground in a stainless steel mill and analysed for Na content by ICP spectrometry. This was undertaken by Waite Analytical Services.

*Na content of the blade of leaf three of thirty lines from the (Kalka#4*Na49) population*

Fifteen F_2 derived lines were selected at random from each of the high and low Na peaks in the distribution of progeny displayed in Figure 5.5. These were sent to R. Munns at CSIRO Plant Industry for analysis of Na concentration in the blade of leaf three, the sheath:blade ratio for Na concentration and the presence of the marker on chromosome 2A. These analyses were undertaken using the methods described by Munns *et al.* (2003).

Of the thirty lines selected, one of the Na lines classified as being a Na excluder was later shown to be a misclassified heterogeneous line after ICP analysis of the material sampled from the Port Pirie field trial.

6.3 Results

*Na uptake of the (Tamaroi*Na49) population at anthesis*

Analysis of variance of Na data of the parental lines Na49 and Tamaroi indicated that genotype was highly significant ($P<0.001$) and that replicate had no significant effect. The mean Na concentration of the Na49 and Tamaroi plants was 773±120 and 9165±310 mg/kg respectively.

The F_2 derived progeny of the cross (Tamaroi*Na49) ranged from 230 to 10800 mg/kg with a mean of 2337 ±2527mg/kg (Figure 6.1). A single peak of approximately fifty-nine individuals occurs at the low concentration end of the distribution (ranging from 230-1480mg/kg), consistent with the level of exclusion observed in Na49. Above 1500mg/kg the progeny are distributed more or less continuously to the Na concentration of the Tamaroi plants. Although the F_2 derived lines were not replicated, the Tamaroi and Na49 replicates indicated that there was good discrimination between the extremes of the distribution. Hence, the very low frequency of normal types recovered by Munns *et al.* (2003), was repeated under the Waite Institute conditions. Munns concluded that only three lines were homogeneous high Na types, with the same Na uptake of Tamaroi. Three apparently homogeneous high Na lines are clearly visible at the high Na concentration end of the distribution in the experiment undertaken at the Waite Campus (Figure 6.1.). Munns has plotted the Na data obtained in this experiment against the data obtained by her own method and allowed it to be reproduced in Figure 6.2. The relationship between the two data sets is not close in the range of Na concentration intermediate to the two parental means, but corresponds closely at the extremes of the distribution.

The three (Na49*Tamaroi) lines identified by Munns *et al.* (2003) as having high Na uptake were the same three identified in the Waite Campus experiment, confirming the low proportion of high Na accumulating lines identified by Munns *et al.* (2003) in their population.

Figure 6.1. The frequency distribution of F_2 derived F_3 lines selected from the cross (Tamaroi*Na49) for Na concentration (mg/kg), grown in the glasshouse at the Waite Campus.

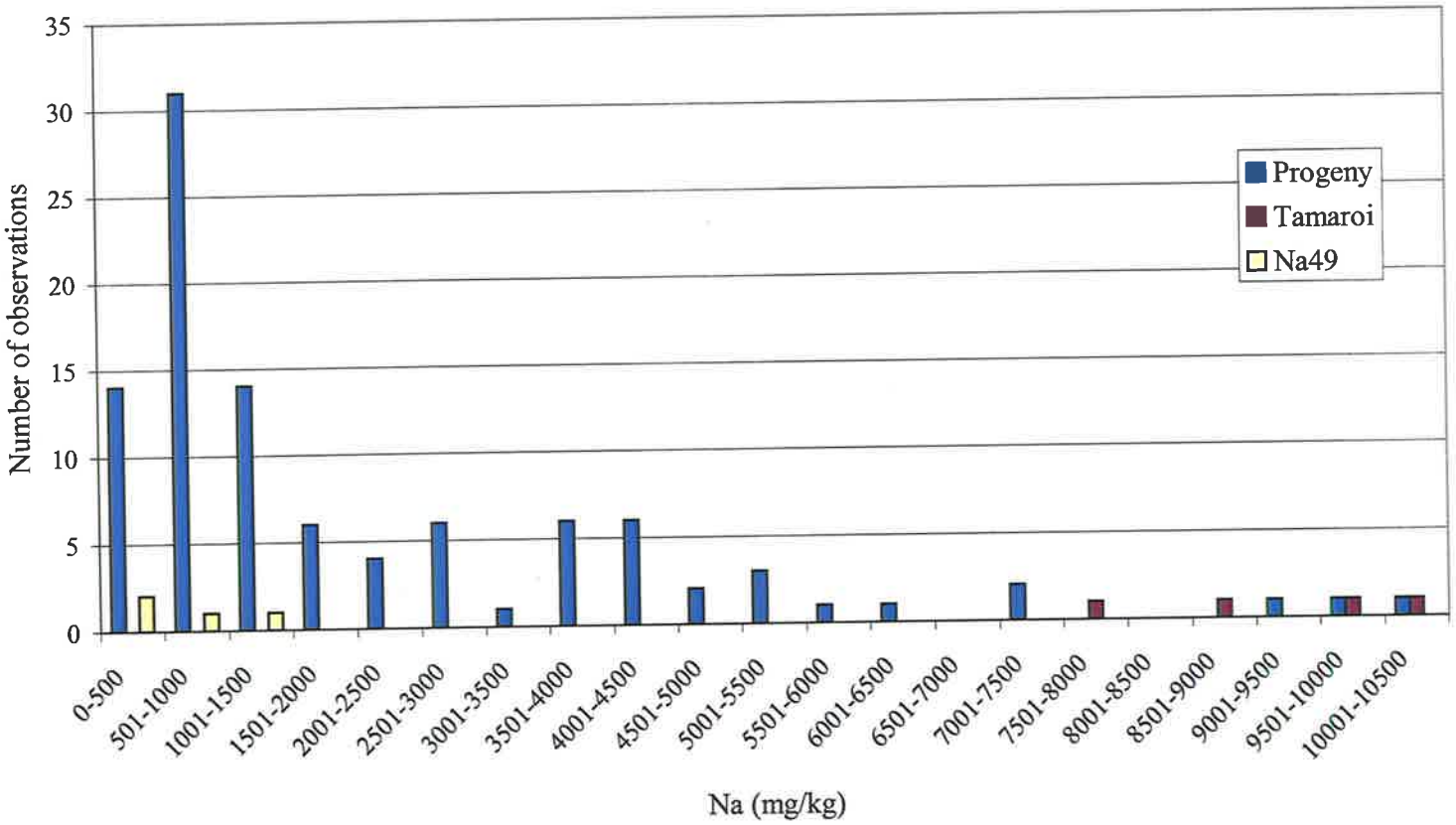
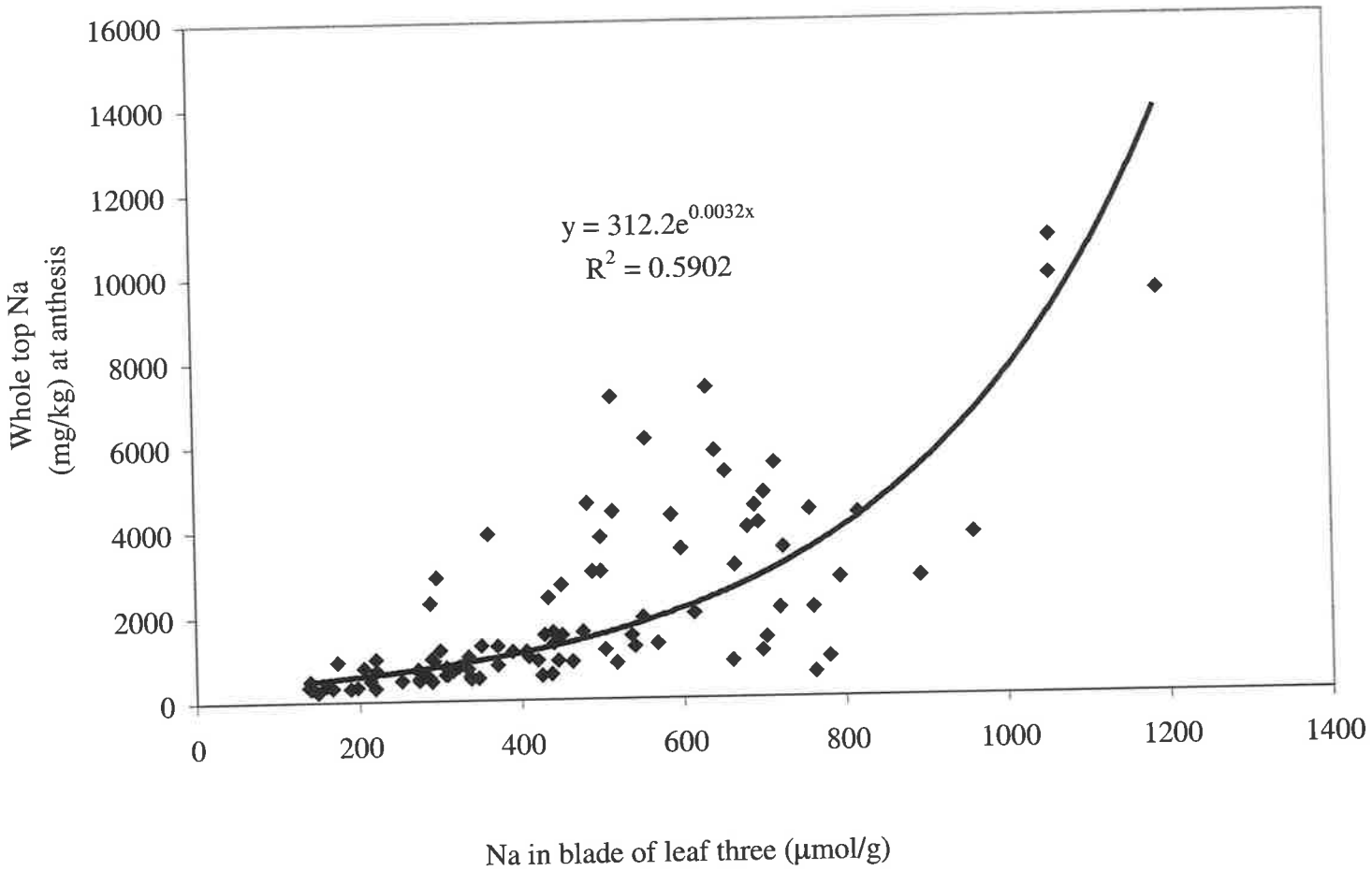


Figure 6.2. Comparison of Na concentration of whole top at anthesis (mg/kg) (Waite campus method) and of the blade of leaf three ten days after emergence from the sheath (µmol/g) (CSIRO method) for F_2 derived F_3 population from the cross (Tamaroi*Na49)



*Na concentration of the blade of leaf three of thirty lines from the (Kalka#4*Na49) population*

All fifteen of the selections that had been shown to be high Na accumulators at anthesis (in Waite Campus experiments) had high Na concentration of the leaf blade consistent with that observed in Tamaroi (Kalka was not included in the test) (Table 6.1). Further, none of these lines had preferential sheath to blade partitioning or the chromosome 2A marker that was hypothesised to be associated with the Na exclusion phenotype.

Of the fourteen low Na selections, all but two (line 93 and 138) had leaf blade Na concentrations consistent with that of Na49 (Table 6.1). Each of these two lines had one replicate that was higher than expected which appeared to be the result of either seed heterogeneity or experimental error. If the two high observations (the expected rep retained in each case) were removed, the mean Na concentration of the low Na group was 196 μmol/g, which was consistent with the mean of Na49.

The fourteen low Na lines also had high sheath:blade ratios (Table 6.1), indicating that the proposed sheath retention trait had co-segregated with Na exclusion. Analysis of Variance of Na concentration in the sheath revealed that Na concentration was significantly higher in the low Na lines than it was in the high Na lines ($P<0.001$), despite the fact that the whole leaf concentration (sheath + blade) was lower. This provides evidence that the Na excluding plants actively sequester Na from the xylem in the leaf sheath to further reduce Na concentration in the leaf blades. Consequently, the high sheath:blade ratio observed in Na49 is not simply the result of reduced leaf accumulation. The marker associated with the sheath retention trait (located on Chromosome 2A) was also present in all but one of the lines (line 39).

Table 6.1. Data from the 15 high and 14 low Na accumulating lines, comparing the results from screening at the University of Adelaide with analysis of leaf blades at CSIRO, Canberra. Na concentration (µmol/g) of the blade and sheath of the third leaf and the ratio of sheath to blade. Results courtesy of R. Munns.

Line	F_2 classification	F_4 classification	Reps	Blade Na	Sheath Na	Sheath + Blade	Sheath : Blade	2A Marker
39	Low	Low	2	216	1042	416	4.8	Absent
93	Low	Low	2	**295φ**	1314	**313**	5.4	Present
95	Low	Low	2	170	853	316	5.0	Present
118	Low	Low	2	170	959	335	5.6	Present
119	Low	Segregating	2	174	1155	460	4.2	Present
138	Low	Low	2	**440φ**	1189	**627**	3.1	Present
147	Low	Low	2	227	972	382	4.4	Present
157	Low	Low	2	204	1043	387	5.4	Present
168	Low	Low	2	169	949	330	5.7	Present
169	Low	Low	2	199	883	350	4.6	Present
177	Low	Low	2	217	963	373	4.4	Present
181	Low	Low	2	179	1195	403	7.2	Present
183	Low	Low	2	184	1034	365	6.0	Present
192	Low	Low	2	181	1036	356	5.7	Present
194	Low	Low	2	257	1105	421	4.7	Present
Average	**Low**			**226**	**1046**	**389**	**5.1**	
1	High	High	2	690	633	679	0.9	Absent
45	High	High	2	793	853	804	1.1	Absent
50	High	High	2	661	783	682	1.2	Absent
51	High	High	2	741	862	764	1.2	Absent
59	High	High	2	756	869	777	1.2	Absent
71	High	High	2	787	728	778	0.9	Absent
76	High	High	2	908	882	903	1.0	Absent
80	High	High	2	570	715	596	1.3	Absent
83	High	High	2	525	763	568	1.5	Absent
113	High	High	2	651	763	647	1.0	Absent
122	High	High	2	606	639	613	1.1	Absent
123	High	High	2	543	646	545	1.0	Absent
124	High	High	2	580	555	593	1.1	Absent
155	High	High	2	563	649	572	1.1	Absent
166	High	High	2	738	610	744	1.0	Absent
Average	**High**			**674**	**768**	**684**	**1.1**	
Na49			4	197	1044	384	5.5	Present
Tamaroi			4	752	980	789	1.3	Absent

Φ Lines 93 (152 and 438µmol/g of Na) and 138 (229 and 652µmol/g of Na) had one replicate each that was higher than expected.

6.4 Discussion

The (Tamaroi*Na49) population segregated in the same manner for whole plant Na concentration at anthesis (Waite method) as it had for the blade of leaf three (CSIRO method), confirming that the trait was not segregating in the 1:2:1 ratio expected for single gene control in this cross (Figure 6.1). This refutes the fifth hypothesis outlined in the introduction (Section 6.1), that two genes have an effect in the juvenile stage of development and that only one of these is important in adult plants.

The presence of the preferential sheath partitioning trait in all of the fourteen low Na selections from the cross (Kalka#4*Na49) provides strong evidence that this co-segregates with the whole plant exclusion locus. If the sheath partitioning trait is controlled by a non-linked second locus, only 6.25% (<1 in 14 tested) of the progeny would be expected to inherit the locus after four cycles of crossing without selection. Hence, it can be concluded that the preferential sheath partitioning trait is either controlled by the same gene as whole plant exclusion, or by a gene very closely linked to it, along with the molecular marker on chromosome 2A. This refutes the fourth hypothesis, that a gene had been lost through backcrossing.

Recent work by Tester (unpublished) has shown that there is no difference in influx into the root between high and low Na accumulating lines; therefore, it is highly likely that efflux is the basic mechanism leading to the low Na concentrations in the shoot and that this results from a restriction on xylem loading, or the promotion of xylem unloading of Na (Tester, pers comm.). Hence, the same gene action, unloading of Na from the xylem, could be responsible for both the lower Na accumulation in the plant and the high sheath/blade Na ratio.

The line that did not have the molecular marker (line 39) has repeatedly had low Na concentrations and in this experiment also had preferential sheath partitioning. This suggests that either a low frequency of recombination occurs between the marker and the Na exclusion locus, or the marker result on this line was incorrect.

The first hypothesis outlined in the introduction was that the locally adapted parent Kalka carried one of the two loci segregating in the (Tamaroi*Na49) population for reduced Na accumulation. While Tamaroi and Kalka have not been included in an experiment that definitely refutes this possibility, the similarities in parentage between them and the limited ICP data available from non-replicated experiments, suggests that this is highly unlikely.

Hypotheses two and three were both based on the large genetic differences between Na49 and Tamaroi. Hypotheses two was that the wide cross results in variation in other traits which indirectly affect the uptake of Na. This was quite likely to have an effect, but was probably not responsible for the magnitude of the differences in Na accumulation resulting from exclusion or sheath partitioning.

Hypothesis three was that the wide cross between Tamaroi and Na49 has resulted in distorted segregation ratios. Presumably, this distortion did not occur in the backcross derived population because the parents used to make the final cross were more closely related. If this was the case, it would be expected that segregation distortion would have been observed in the progeny arising from earlier cycles of crossing in the development of the BC_3 derived population (Kalka#4*Na49). This did not occur, even in the BC_1 progeny (see Figure 5.1 of Chapter 5), suggesting that any segregation distortion is particular to the cross between Tamaroi and Na49. Kammholz *et al.* (2001) found that segregation

distortion occured in distinct sections of chromosomes, but that homologous sections did not exhibit distorted ratios in other crosses. This suggests that it was quite possible that segregation distortion occurs in the cross between Na49 and Tamaroi, but not in the cross with Kalka.

The segregation ratios observed at all stages of the backcrossing process (Chapter 5), without loss of either preferential sheath partitioning or whole plant exclusion, repeatedly provided robust evidence for co-segregation of the two traits at a single locus. In contrast, the conclusion reached by Munns *et al.* (2003) that two dominant genes were responsible was principally based on the fact that approximately only 1 in 16 high Na selections were recovered in the F_2 generation. Beyond this segregation ratio, the evidence for two gene control is not strong. Firstly, there is no evidence of distinct classes in the distribution of progeny for Na concentration and secondly, too few F_2 derived F_3 families were tested toaccurately assess the numbers of homozygous and heterozygous families. If F_3 families, derived from intermediate F_2 plants, had been tested and some shown to not segregate, this would have provided irrefutable evidence that partial Na exclusion was conferred by a single gene, supporting the two gene theory.

In conclusion, the two-gene theory of Munns *et al.* (2003) is probably incorrect. This theory was based primarily on the low proportion of high Na accumulating parentals recovered in the F_2 progeny. One of two hypotheses were likely to be responsible for the distorted segregation ratios observed. The first was that segregation distortion occurred due to genetic incompatibilities between Na49 and Tamaroi. The second hypothesis was that one or more of the F_1 plants that the F_2 seeds were selected from was the result of self pollination.

Chapter 7.

THE EFFECT OF Na EXCLUSION ON GRAIN YIELD AND GRAIN SIZE OF DURUM WHEAT

7.1 Introduction

The results described in Chapter 5 have shown that the genetic material controlling Na exclusion from the landrace Na49 results in a large and significant reduction in Na accumulation in the whole plant tops. This trait is controlled by a single dominant gene, which has been transferred through backcrossing into the background of a locally adapted genotype, Kalka.

Commercial durum varieties have been shown repeatedly to be more intolerant of salinity than bread wheat (Francois *et al.* 1986; Rawson *et al.* 1988). Munns *et al.* (2000) proposed that the difference in tolerance was due to the greater ability of bread wheat, compared to durum, to exclude Na from the plant shoot.

Many authors have proposed reduced Na accumulation in the plant shoot as a mechanism of tolerance (Flowers *et al.* 1977; Greenway and Munns 1980; Chhipa and Lal, 1995), while Maathuis and Prins (1990) reported that salt tolerant *Plantago maritima* accumulated lower concentrations of Na than the intolerant species *Plantago medica*. In an attempt to identify *Triticum turgidum* with enhanced Na exclusion, Munns *et al.* (2000) screened sixty-four genotypes including accessions of the *T. turgidum* subspecies, *durum*, *turgidum*, *polonicum, turanicum and carthlicum*. This work identified the durum accession Na49 as having a high level of Na exclusion and it was proposed that this line could be used as a donor parent for improving the salt tolerance of commercial durum varieties.

Dvorak *et al.* (1994) attempted to improve the Na exclusion of durum wheat by transferring the *Kna1* locus of bread wheat into a durum background by homoeologous recombination of the long arm of chromosome 4D with 4B of the durum variety 'Langdon'. A series of 4D recombinants were produced; nine carrying *Kna1* and fifteen without. When the lines where grown under saline conditions the recombinants with *Kna1* had significantly more biomass, but no significant difference in grain yield was measured.

Many different methods, which range in accuracy and reputability, have been used for comparing the effect of specific genetic differences. At the least convincing end of the scale, comparisons have been made between species, while other workers have investigated the differences between individual, unrelated genotypes. Both of these comparisons do not take into consideration the effect of the many genetic differences between the genotypes, which are completely unrelated to the gene/s of interest, but which also contribute to the phenotype.

A far less ambiguous method is to compare groups of material, which differ for the alleles of interest (Na exclusion), but have equivalent genetic backgrounds (Snape,1987). An equivalent genetic background is one where there is a reasonable level of confidence that the observed differences are due to the effect of the major gene/s under investigation and do not arise from spurious background genetic effects. Isogenic lines and random lines arising from a single hybridisation are the most suitable genetic material on which to make these comparisons (Snape, 1987).

Isogenic lines are produced by crossing the recurrent parent with the donor parent (carrying the trait of interest) and then proceeding through at least five backcrosses to the

recurrent parent. Single plants of the alternative genotypes are selected from the progeny of a heterozygous plant arising from the last backcross. Lines are developed from these plants and co-variance is studied between the trait of interest and other characters (Snape, 1987). To produce isogenic lines, the trait must be under major gene control to facilitate selection of the donor trait between each round of backcrossing. If the mode of genetic control is not known, the most suitable option is the random line approach. In this approach two contrasting genotypes are crossed and a set of recombinant lines are selected from a segregating generation; normally the F_2 (Snape, 1987). Because the population will also be segregating for a large amount of unrelated genetic variation arising from the parental genotypes, the random population should comprise at least 100 and preferably 200 F_2 derived lines (Rathjen pers comm.). An alternative to the random line approach is to produce doubled haploid lines from the cross between the two genotypes.

Despite the international interest in salinity and the quantity of literature proposing Na exclusion as a mechanism of salt tolerance, there has been no study published that adequately investigates the effect of reduced Na accumulation in a population with equivalent background. The work of Dvorak *et al.* (1994), described earlier in this introduction, is one of only two studies to compare the yield of lines derived from a single hybridisation and segregating for Na uptake. This work was based on a population size of only twenty-four homoeologous recombinants, nine containing the *Kna1* locus and fifteen without. A second study by Husain *et al.* (2003) compared two Na excluding and non-excluding F_2 derived lines from the cross between Na49 (Na excluding) and Na41 (non-excluding) in solution culture. These two studies both recorded statistically significant differences between the Na excluding and non-excluding groups, but with such low population sizes, it is impossible to be sure that background genetic effects were not

responsible. With adequate population sizes (in excess of 100), the results of these two studies would have had far greater validity.

The population developed from the third backcross to Kalka (Chapter 5) was selected from single F_2 plants; therefore it was intermediate to the two types of material described by Snape (1987). The backcross method was used to reduce the genotypic contribution of the unadapted donor parent Na49 and proceeding through another two backcrosses would have produced lines nearer to isogenic. This improved the ability to measure the effects of the Na exclusion gene, particularly in field trials, in the absence of major genetic background effects. Further backcrossing was not possible in the time allocated for this project and consequently the 195 random lines were selected in the BC_3F_2. The relatively large number of selections was taken to randomise the background effects, enabling the calculation of a relatively robust estimate of the mean effect of the alleles.

This population provides the material for confident assessment of the pleiotropic effects of the Na exclusion gene on other variables, such as yield, grain size and the uptake of other nutrients. When grown on a saline site, a positive response in grain yield or grain size associated with the presence of the gene, will provide supporting evidence for the theory that the trait improves the salt tolerance of durum wheat.

The poor adaptation of Na49 to Australian farming conditions means that its contribution to varietal improvement will be restricted to use as a donor parent in backcrossing programs. The success of any backcrossing program (disregarding unwanted pleiotropic effects) is dependent on a number of backcrosses to reduce the amount of undesirable genetic material contributed by the donor parent, both distributed at random and associated

with linkage drag. The magnitude of the genetic differences between the donor and recurrent parents, combined with the closeness of linkage between the gene of interest and unwanted loci, determine the number of backcrosses and population size needed to incorporate the Na exclusion gene into the adapted background of the recurrent parent. Experience at the Waite Campus suggests that often another series of crossing and selection is required before the major gene of interest has been incorporated into a suitable genetic background and it becomes of major commercial significance.

The population derived form the cross (Kalka#4*Na49) was large enough (186 lines in field trials) to provide an unambiguous assessment of the pleiotropic and linkage effects associated with the Na exclusion allele.

7.2 Materials and methods

The field experiments (described in Chapter 5, Section 5) grown at Two Wells, Port Pirie and Redhill were sown using the machinery and methods of the Waite durum breeding program. These methods are described in detail in Chapter 3.

During the growing season, all of the 186 BC_3F_2 derived lines included in field experiments were screened for the presence of the boron tolerance allele of Kalka, which originates from the Aus#14010 source. This tolerance was shown by Jamjod (1996) to be controlled by a single locus (Bo_T2) on chromosome 7B.

The lines were tested in two separate groups in the solution culture method described by Campbell *et al.* (1998), with each line represented by six BC_3F_2 derived F_4 seeds from the multiplication rows grown in the bird proof enclosure. Three replicates of three seeds were

included of each of the parental genotypes, Kalka and Na49, in both experiments. To standardise the variation between the experiments, the root lengths were expressed as a percentage of the experimental mean.

The BC_3F_2 derived lines were scored for stage of development at Port Pirie on the 10th of September and Redhill on the 10th of October on a scale of one to five, where one was very early and five, very late. Variation in maturity was evident across the BC_3F_2 derived population, ranging from a maturity time slightly earlier than Kalka (Kalka = 3), to lines that matured in excess of two weeks later than Kalka (5). The Two Wells experiment was not scored and consequently, maturity data is not available on the lines that were included in this experiment alone.

The Port Pirie and Redhill experiments were harvested on the twenty-fourth of November and second of December respectively, followed by the Two Wells, on the fourth of December. The individual grain samples were cleaned and sieved through a Carter-Day® Dockage Tester using a 2mm screen. The portion of grain passing through the screen was weighed and the screenings percentage calculated.

7.3 Results

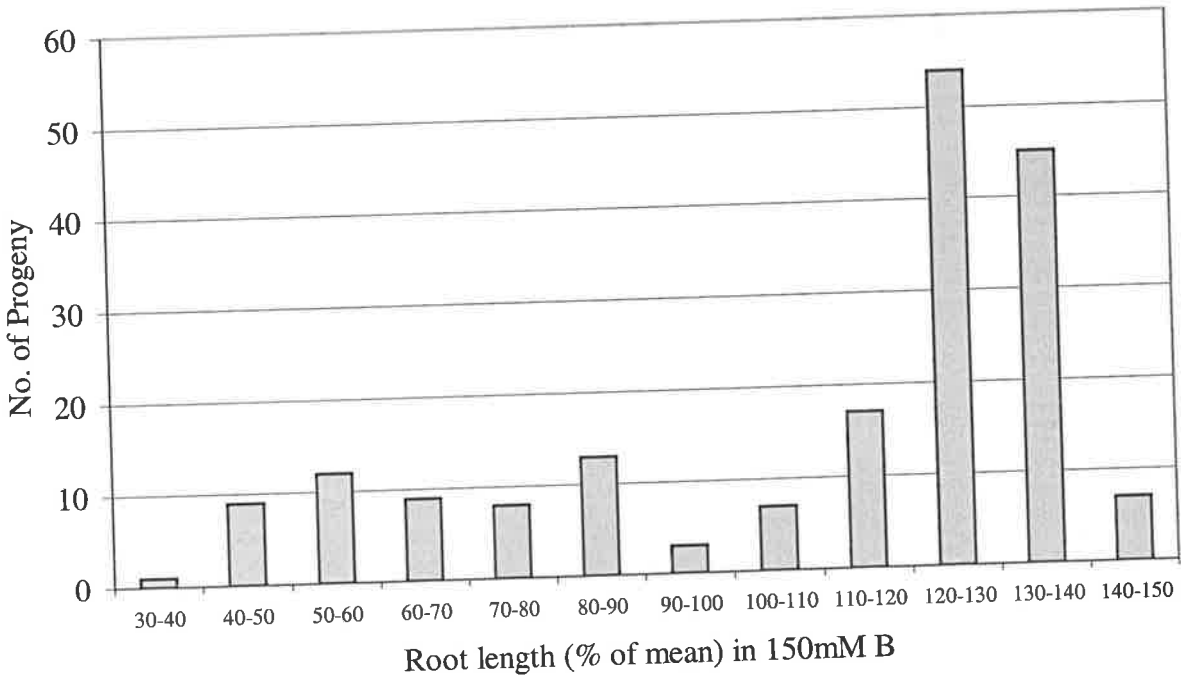
Boron tolerance of the 186 BC_3F_2 derived lines included in field experiments

Analysis of variance of the six replicates (includes three from each experiment) of the parental genotypes (Kalka and Na49) indicated that there was no significant effect of replicate and that genotype had a significant ($P<0.001$) effect on the root length of seedlings grown in boron toxic solution. The mean root lengths (% of experiment mean) of

Kalka and Na49 were 123 ±13.5% and 49.9 ±8% respectively, compared to the experimental mean of 100 ±31%.

The non-significant replicate effect allowed the results of the two experiments to be combined and consequently, the distribution of BC_3F_2 derived lines (Figure 7.1) includes all lines from both experiments.

Figure 7.1. Frequency distribution of seedling root length (mm) of 186 BC_3F_2 derived lines of the cross (Kalka#4*Na49) grown in 150mM B solution for 10 days. Root length expressed as % of experiment mean.



The large peak at the high end of the distribution (in the range 120-140%), was consistent with the mean of 123% obtained for the boron tolerant recurrent parent Kalka. This peak corresponded to the homogeneous boron tolerant progeny. A low frequency of progeny occurred between 90 and 100%, below which the variation was more or less continuous. If the 95% level separates the homogeneous tolerant lines from the heterogeneous and

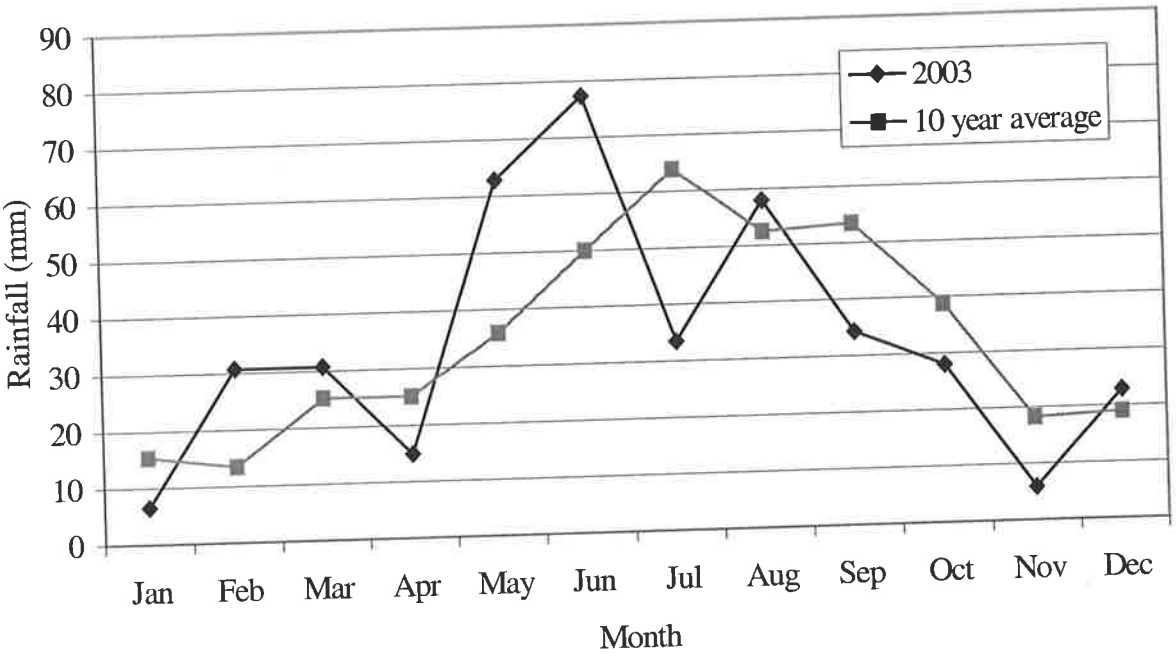
homogeneous intolerant lines, then 132 of the 186 lines were homogeneous boron tolerant. Probably, the two points of highest frequency (50-60 and 80-90%) below the 95% level were consistent with the means of the homogeneous intolerant and heterogeneous lines respectively. Without a characterisation of the BC_2F_1 parents, it is not possible to formulate expected numbers in the homogeneous and heterogeneous classes.

Grain Yield

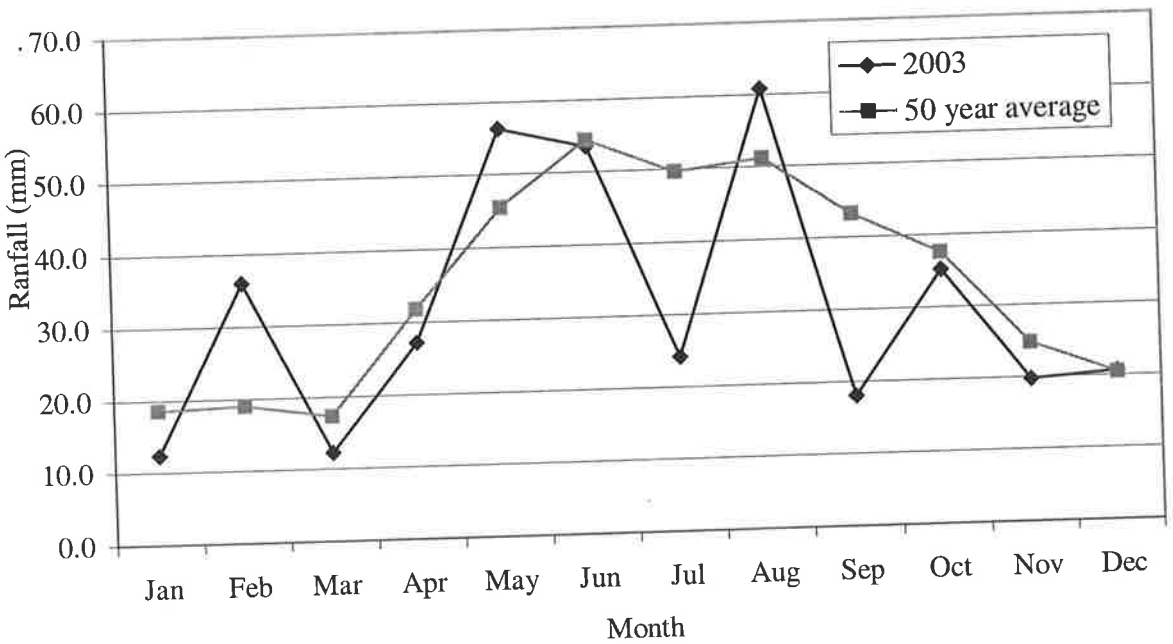
One of the key environmental factors affecting the yield and screenings results obtained from the field experiments was the drier than average spring experienced in 2003. This could be expected to place a very large reliance on subsoil moisture for grain fill, which should have increased any benefit associated with tolerance to subsoil constraints. The comparison between the monthly rainfall for 2003 and the long-term average (Figure 7.2) indicates that all three sites had below average September and October rainfall (there has also been speculation that grain yields of wheat in 2003 were adversely affected by two periods of exceptionally high air temperatures).

Figure 7.2. Total monthly rainfall (mm) recorded in 2003, compared to long-term average data for, (a) Two Wells; (b) Redhill; and (c) Port Pirie. Data provided by Mr. Neville Sharpe, Mr. John Wheaton and Mr. Philip Johns respectively.

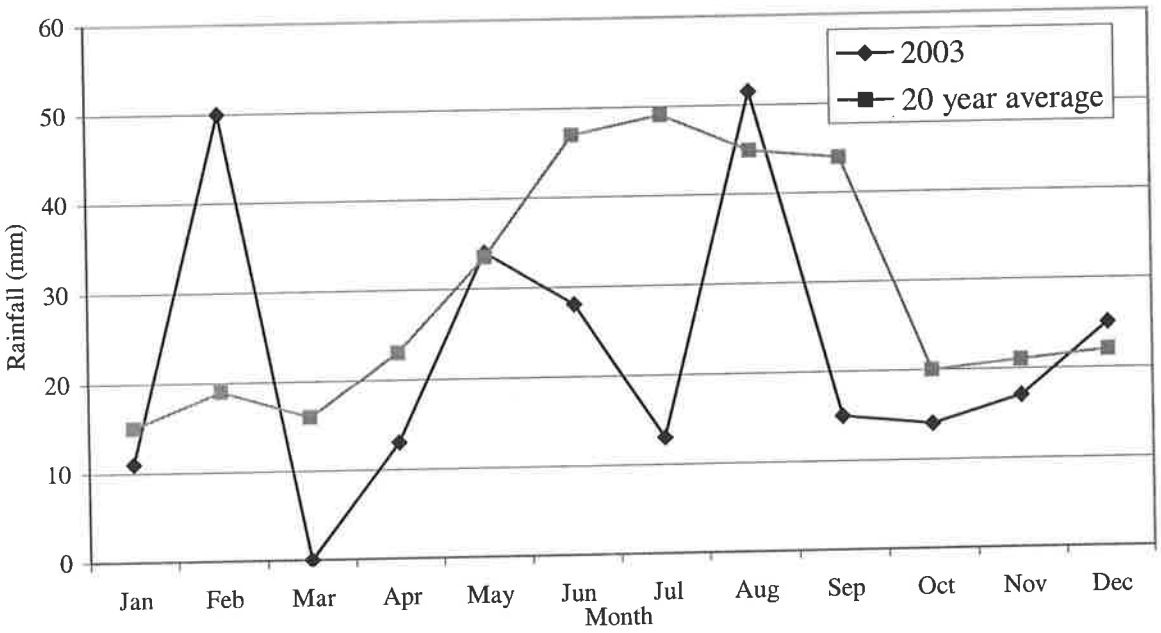
(a) Monthly rainfall (mm) recorded at Two Wells.



(b) Monthly rainfall (mm) recorded at Redhill.



(c) Monthly rainfall (mm) recorded at Port Pirie.



The mean grain yields of all plots harvested at the Two Wells, Redhill and Port Pirie sites were 166 ±110, 681 ±136 and 55 ±56 g/plot respectively, which was approximately equivalent to, 0.55, 1.82 and 0.18 t/ha. The mean yields of the Kalka and (Kalka#*Na49)/2/85 plots are tabulated in Table 7.1.

Table 7.1. Grain yield (g/plot) of Kalka and (Kalka#*Na49)/2/85 check plots at the 2003 field sites.

	Kalka	(Kalka#*Na49)/2/85
Two Wells	170 ±117	167 ±115
Redhill	730 ±118	425 ±80
Port Pirie	60 ±63	38 ±32

Correlation coefficients were calculated for the relationships between the grain yields and the three traits for which the population had been characterised (Table 7.2), which were, Na concentration (mg/kg) in whole tillers, root length in boron toxic solution and maturity scored on a 1-5 scale (1 being early and 5 late).

Table 7.2. Correlation coefficients (r) for the relationships between grain yield at three locations and other traits of the BC_3F_2 derived population from the cross (Kalka#4*Na49).

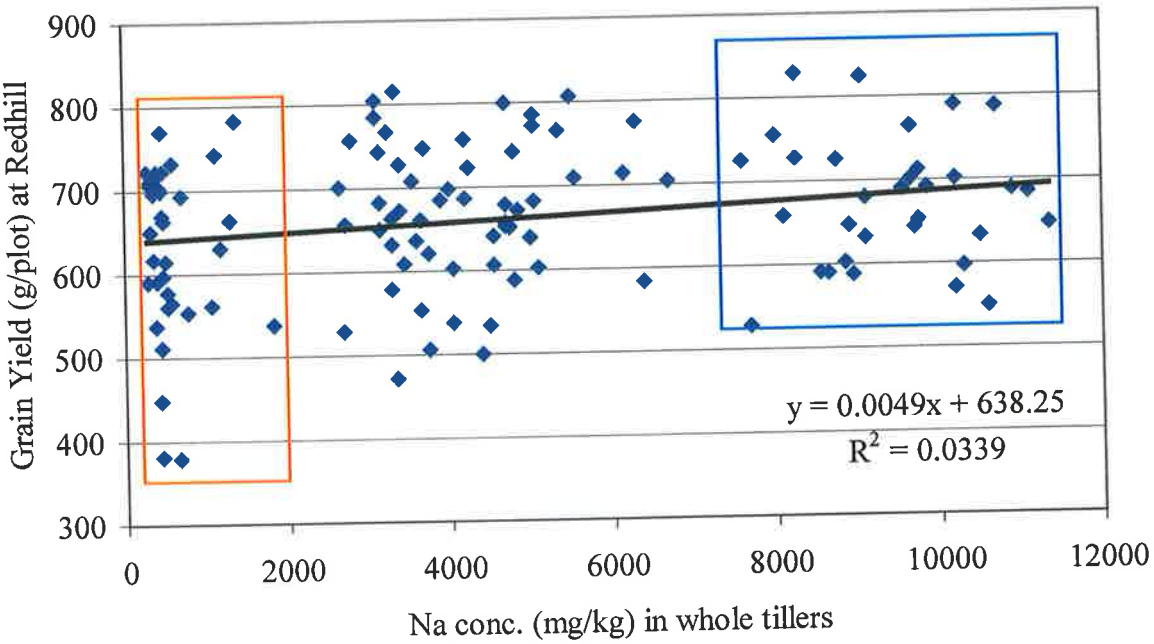
	Na concentration	Root length in B	Maturity
Two Wells	-0.29***	0.25***	-0.35***
Redhill	0.18*	-0.08 ns♣	-0.49***
Port Pirie	-0.13 ns	0.33***	-0.31***

♣ not significant; * (P<0.05); ** (P<0.01); *** (P<0.001)

Redhill

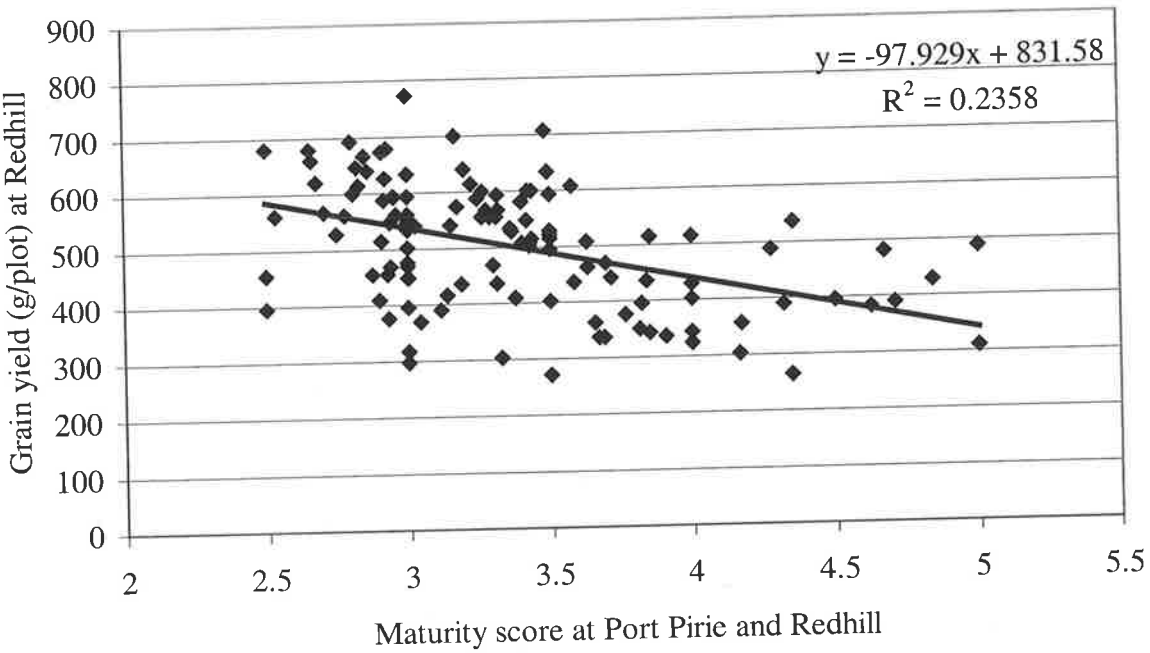
The Redhill site achieved the highest yield and was expected to provide the best measure of the tolerance to transient salinity conferred by Na exclusion under typical durum production conditions. Unexpectedly the correlation coefficient for the relationship between Na accumulation and yield was 0.18, indicating that the Na excluding lines had yielded less than the high Na accumulating 'normal' types (Figure 7.3). This relationship was demonstrated by the ninety homogeneous excluding lines having a mean yield of 631 ±102 g/plot, compared with 676 ±76 g/plot for the 126 homogeneous non-excluders, a 7% difference in yield.

Figure 7.3. The relationship between Na concentration in whole tillers sampled at Port Pirie and the grain yield at Redhill of BC_3F_2 derived lines selected from the cross (Kalka#4*Na49). Lines inside the red box are homogeneous Na excluding lines, those inside the blue box are homogeneous non-excluding lines and those outside of both are heterogeneous.



The root length of seedlings grown in boron toxic solution culture had a non-significant correlation coefficient of –0.08 with grain yield. Maturity score on the other hand had a significant correlation ($P<0.001$) with yield, with a coefficient of 0.49 (Figure 7.4). The correlation between maturity and Na concentration ($r = 0.016$) in whole tillers was not significant, which indicated that the low Na lines did not have lower yields as a result of having inherently late maturity.

Figure 7.4. The relationship between maturity score (1 = early, 5 = late) and grain yield at Redhill of the BC_3F_2 derived lines selected from the cross (Kalka#4*Na49).



Port Pirie

The Port Pirie site was affected by a severe infection of crown rot (*Fusarium pseudograminearum*) and consequently, the mean yield was only 55 ±56 g/plot, which is equivalent to about 0.2t/ha.

The correlation coefficient of -0.13, calculated for the relationship between the grain yield of the BC_3F_2 derived lines and the Na concentration of whole tillers sampled from Port Pirie, was not significant. On the other hand, the relationships between boron tolerance (root length in B toxic solution) and grain yield, and maturity and grain yield were both significant ($P<0.001$), with correlation coefficients of 0.33 and –0.31 respectively (Figures 7.5 and 7.6).

Figure 7.5. The relationship between root length of seedlings grown in B toxic solution for ten days and grain yield at Port Pirie of 164 BC_3F_2 derived lines selected from the cross (Kalka#4*Na49).

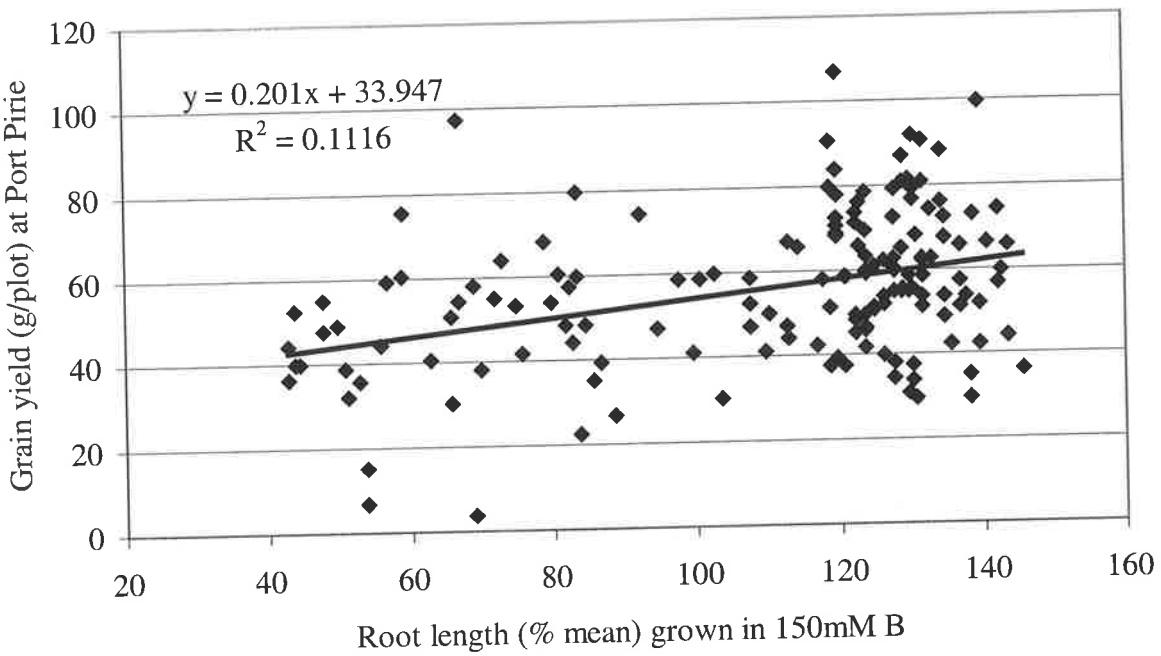
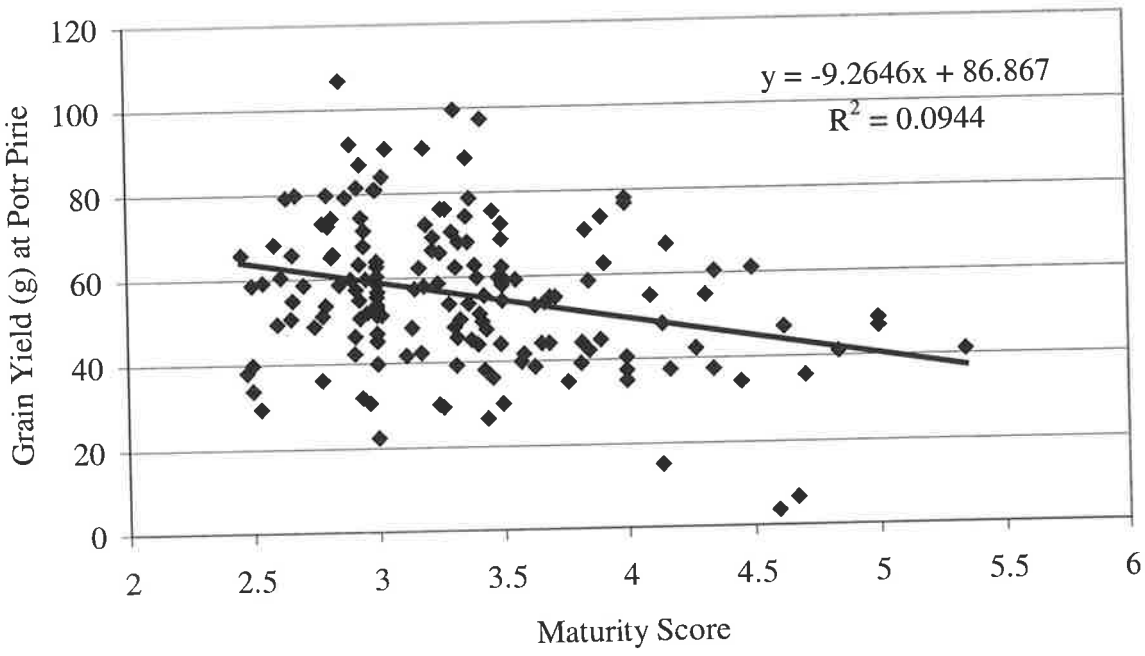


Figure 7.6. The relationship between maturity score (1 = early, 5 = late) and grain yield at Port Pirie of 164 BC_3F_2 derived lines selected from the cross (Kalka#4*Na49).



Two Wells

The mean grain yield harvested at Two Wells was only 166 ±110 g/plot, which is approximately equivalent to 0.55t/ha. This low grain yield resulted from the hot and dry conditions that occurred at the end of September and in November, and was accentuated by the later than optimal seeding date. Consequently, any tolerance to the subsoil toxicities which occurred at this site was expected to have had a large impact on grain yield through increased access to subsoil water. Kalka plots in an adjacent experiment sown on the fourth of June (21 days earlier) averaged 2.1 tonne/ha and had satisfactory grain finish.

The correlation coefficient of -0.29, calculated for the relationship between the grain yield of the BC_3F_2 derived lines at Two Wells and the Na concentration of whole tillers sampled in the field, was significant ($P<0.001$). This indicated that the Na excluding lines were higher yielding than the high Na accumulating 'normal' types (Figure 7.7) and this resulted in the homogeneous Na excluders yielding 181 ±67 g/plot compared to 143 ±77 g/plot for the homogeneous non-excluders.

The relationship between boron tolerance and grain yield was also significant ($P<0.001$), with a correlation coefficient of 0.25, indicating that boron toxicity affected grain yield at the Two Wells field site (Figure 7.8). This was also the case for maturity, which had a significant ($P<0.001$) correlation coefficient of -0.35 with grain yield (Figure 7.9).

Figure 7.7. The relationship between Na concentration in whole tillers sampled in the field (Port Pirie and Two Wells) and the grain yield at Two Wells of 186 BC_3F_2 derived lines selected from the cross (Kalka#4*Na49). Lines inside the red box are homogeneous Na excluding lines, while the lines inside the blue box are homogeneous non-excluding lines and the lines outside of both are heterogeneous.

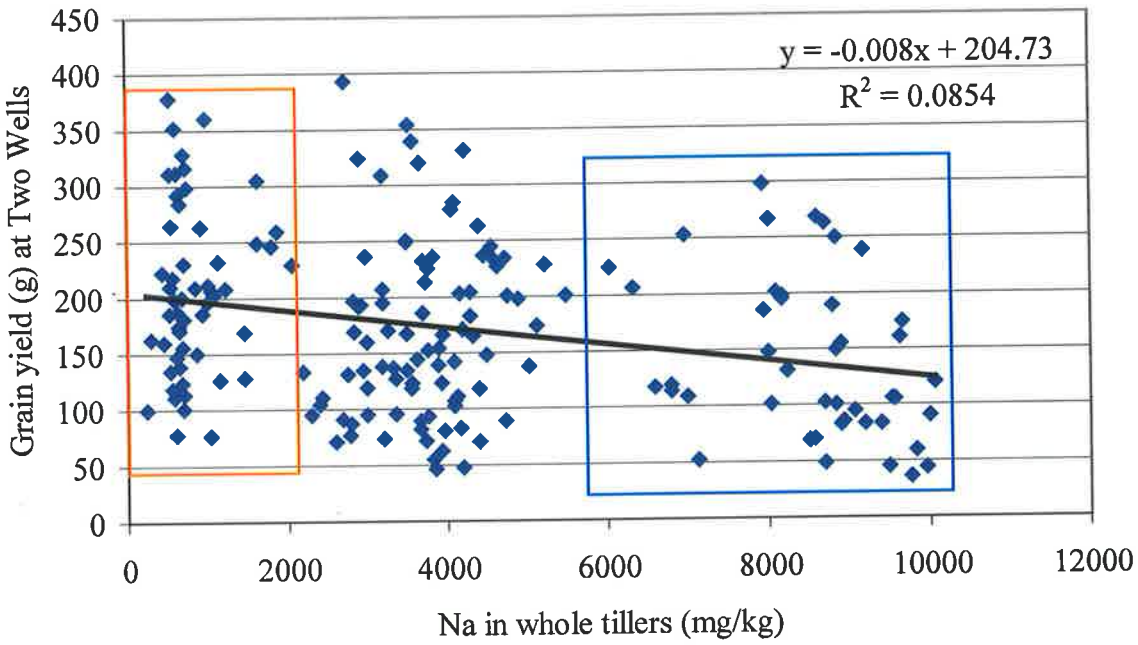


Figure 7.8. The relationship between root length of seedlings grown in B toxic solution for ten days and grain yield at Two Wells of 186 BC_3F_2 derived lines of (Kalka#4*Na49).

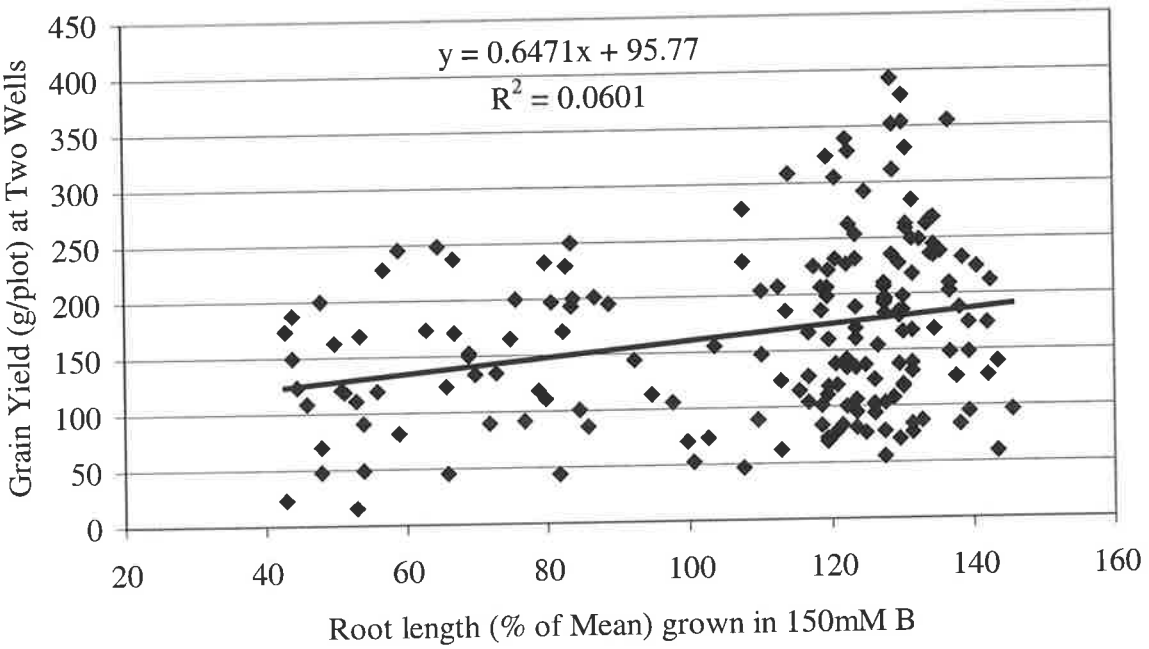
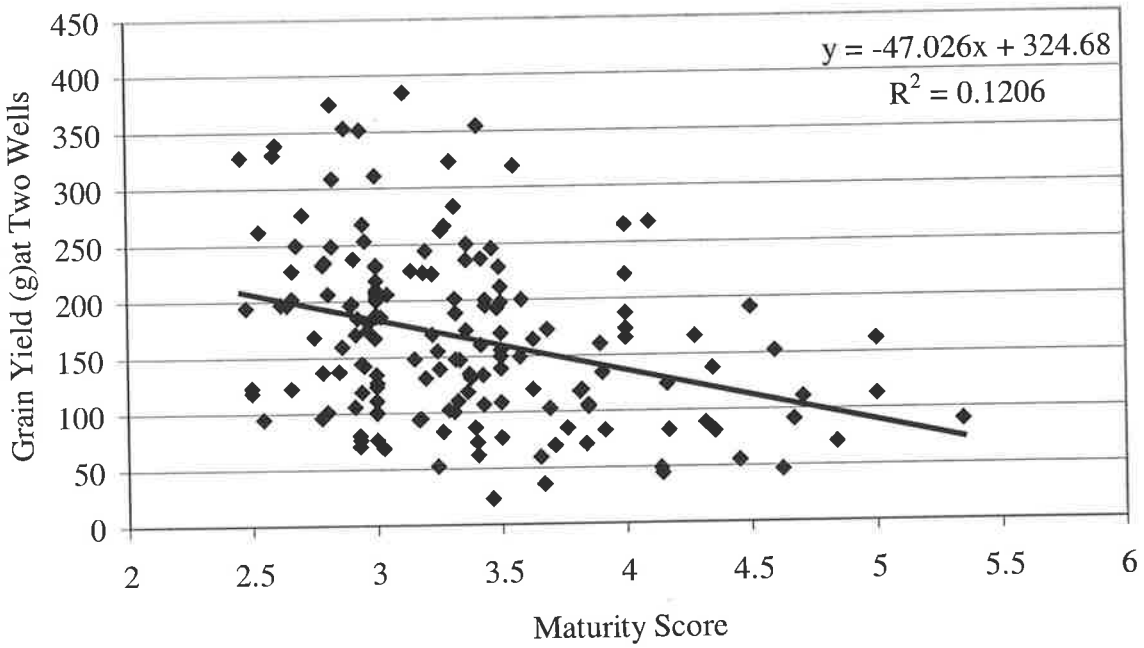


Figure 7.9. The relationship between maturity score (1 = early, 5 = late) and grain yield at Two Wells of 186 BC_3F_2 derived lines selected from the cross (Kalka#4*Na49).



The increase in yield associated with Na exclusion and boron tolerance at Two Wells resulted in a yield increase of 108% in the lines having both Na exclusion and B tolerance (Table 7.3).

Table 7.3. Grain yield (g/plot) of BC_3F_2 derived lines from the cross (Kalka#4*Na49) segregating for boron tolerance and Na exclusion at Two Wells.

Na	B	Yield
Non-excluding	Intolerant	90
Non-excluding	Moderately Tolerant	161
Excluding	Intolerant	162
Excluding	Moderately Tolerant	188

Residual Variation

At each site, there was a large amount of residual variation, unexplained by the relationship between grain yield and each of the significant traits. Was this residual variation due to minor background effects (both genetic and environmental), or segregation of one, or more major genes affecting yield? In an attempt to gain more information on this, multiple linear regression analysis was undertaken to determine the equation best describing yield from the known variables (maturity, boron tolerance and Na exclusion) affecting yield. If the residual variation resulted from the segregation of minor genes, the distribution of residuals around the line of best fit would be normal, whereas, if the variation is due to a major gene, the distribution of residuals would not be normal.

At all three sites the residuals were normally distributed, suggesting that the residual variation was due to multiple genes in each case. Correlation coefficients were calculated

for the relationships between the residual variation in yield at the three field sites (Table 7.4). It was found that significant correlations (P<0.05) did occur between two of the three pairs of sites, indicating that segregation has occurred for unidentified traits which do have a significant effect on yield across sites.

Table 7.4. Correlation coefficients (r) for the relationships between residual variation in grain yield across the three field sites.

	Redhill	Port Pirie
Two Wells	0.28*	0.177*
Redhill		0.15 ns♣

♣ ns; * (P<0.05)

Screenings

The mean screenings percentage (<2.0mm) in grain yield harvested from the Two Wells, Redhill and Port Pirie sites were 29.8 ±13.3, 8.3 ±4.0 and 20.4 ±10.9. The mean screenings percentages of the Kalka and (Kalka#*Na49)/2/85 plots are tabulated in Table 7.5.

Table 7.5. Screenings (%) in grain of Kalka and (Kalka#*Na49)/2/85 check plots at the three 2003 field sites.

	Kalka	(Kalka#*Na49)/2/85
Two Wells	30.3 ±13.2	25.7 ±9.7
Redhill	6.5 ±3.9	8.8 ±2.8
Port Pirie	18.1 ±10.1	29.3 ±18.1

Correlation coefficients were calculated for the relationships between screenings (%) and the three traits (Na concentration, root length in boron toxic solution; and maturity relative to Kalka) for which the population had been characterised (Table 7.6).

Table 7.6. Correlation coefficients (r) for the relationships between screenings (%) and other traits (Na concentration (mg/kg) in whole tillers, root length in boron toxic solution and maturity scored on a 1-5 scale) in the BC_3F_2 derived population from the cross (Kalka#4*Na49).

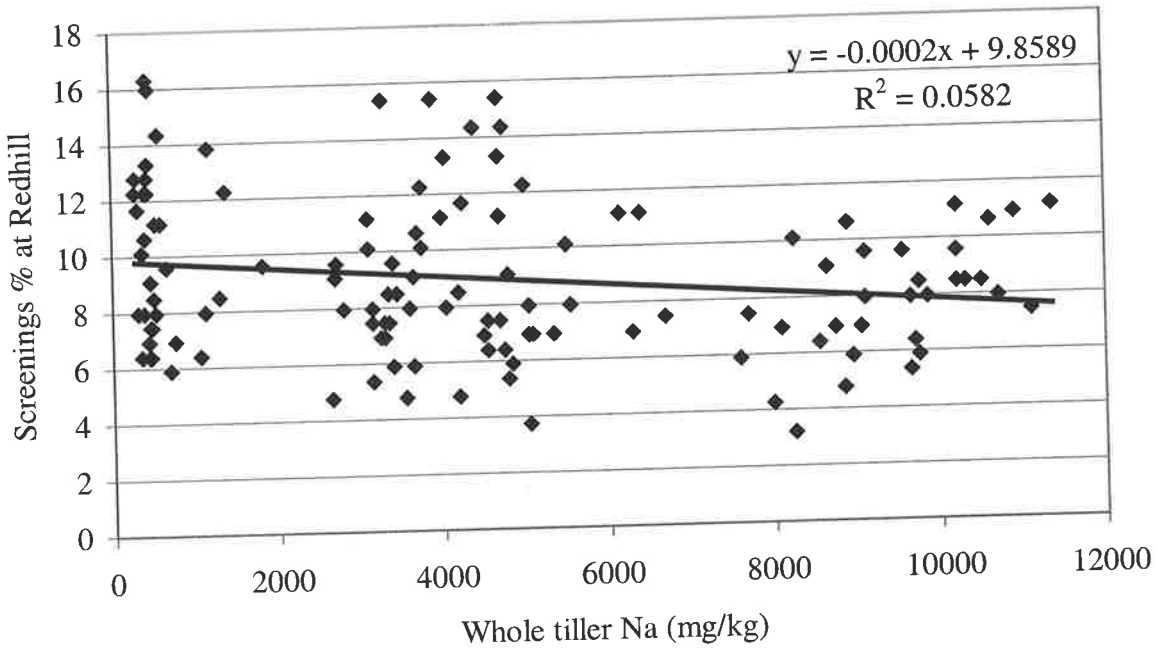
	Na concentration	Root length in B	Maturity
Two Wells	0.28***	0.11 ns♣	-0.02 ns
Redhill	-0.24***	-0.41***	0.58***
Port Pirie	-0.11 ns	0.02 ns	-0.08 ns

♣ not significant; * (P<0.05); ** (P<0.01); *** (P<0.001)

Redhill

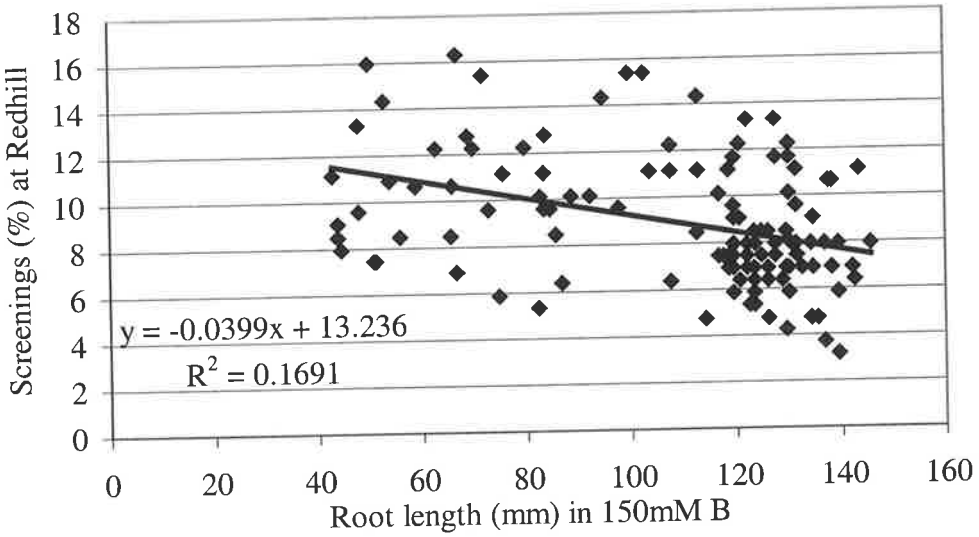
The correlation coefficient of –0.24, calculated for the relationship between the screenings of the BC_3F_2 derived lines at Redhill and the Na concentration of whole tillers sampled from Port Pirie, was significant (P<0.001), indicating, unexpectedly, that the Na excluding lines had smaller grain size than the high Na accumulating 'normal' types (Figure 7.10). This relationship resulted in the homogeneous Na excluding lines having a mean screenings percentage of 10.1 ±2.9%, compared with 7.9 ±2.1% in the grain harvested from the homogeneous non-excluders.

Figure 7.10. The relationship between Na concentration in whole tillers sampled at Port Pirie and screenings (%) at Redhill of BC_3F_2 derived lines selected from the cross (Kalka#4*Na49).



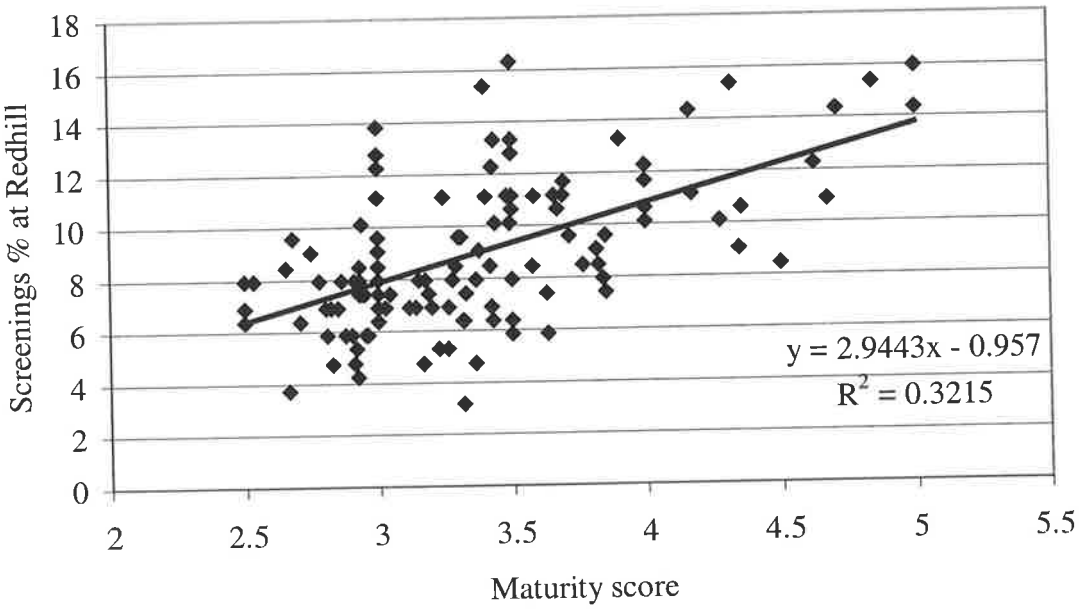
The root length of seedlings grown in boron toxic solution was significantly correlated (negatively) with screenings ($P<0.001$), indicating that boron tolerant lines did have fewer screenings than intolerant lines (Figure 7.11), despite the lack of a significant correlation between grain yield and boron tolerance.

Figure 7.11. The relationship between root length of seedlings grown in B toxic solution for ten days and screenings % at Redhill of BC_3F_2 derived lines selected from the cross (Kalka#4*Na49).



As expected, the terminal drought resulted in maturity also being significantly correlated ($P<0.001$) with screenings (Figure 7.12).

Figure 7.12. The relationship between maturity score (1 = early, 5 = late) and screenings (%) at Redhill of BC_3F_2 derived lines selected from the cross (Kalka#4*Na49).



Two Wells

The terminal drought, combined with transient salinity resulted in a significant correlation ($P<0.001$) between Na accumulation and screenings percentage at Two Wells (Figure 7.13), so the grain of the homogeneous Na excluding lines had a screenings percentage of 26.0 ±7.4 compared with 33.9 ±8.8 for the homogeneous non-excluders. The curtailment of grain fill that resulted in the high percentage of screenings at Two Wells, also resulted in very poor fill of grains that were larger than 2mm and consequently, lower mean weight per grain (Plate 7.1).

Figure 7.13. The relationship between Na concentration in whole tillers and screenings (%) in grain harvested from Two Wells of BC_3F_2 derived lines selected from the cross (Kalka#4*Na49).

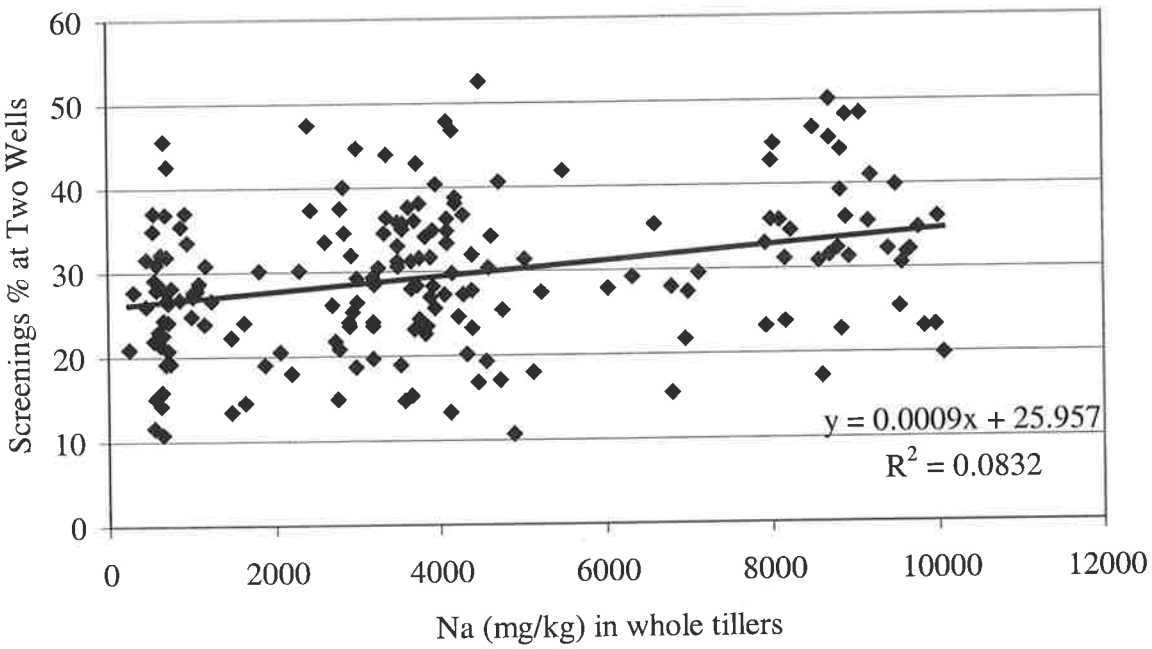


Plate 7.1. A sub-sample of the bulked grain (>2mm) harvested from Kalka plots at Two Wells, 2003.

As was observed with the relationships between the significant traits and grain yield, a large amount of residual variation was unexplained by the relationships between screenings and each of the significant traits. Multiple Linear Regression analysis was caculated to investigate the residual variation at each site in the same way as it was for grain yield.

At all three sites the residuals were normally distributed, suggesting that the residual variation was not due to a single major gene. Correlation coefficients were calculated for the relationships between the residual variation in screenings at the three field sites (Table 7.7), but none were significant.

Table 7.7. Correlation coefficients (r) for the relationships between residual variation in screenings (%) across the three field sites.

	Redhill	Port Pirie
Two Wells	0.01 ns♣	0.06 ns
Redhill		0.06 ns

♣ not significant

7.4 Discussion

The ability to exclude Na, boron tolerance and maturity differences had varying affects at the three field sites, all of which were exacerbated by a drier than average spring. The dry conditions are likely to have increased the reliance on subsoil moisture and consequently, increased the benefit of genetic tolerance to subsoil toxicities.

The Port Pirie experiment had very low grain yields due to a severe infection of crown rot (*Fusarium pseudograminearum*). The most obvious symptom of this disease was premature plant death under conditions of water stress, as fungal invasion is enhanced in stressed tissue (Cook and Christen, 1976). This often results in a random distribution of dead heads throughout the crop, but in the case of the Port Pirie experiment, the death of nearly all of the plants. The only significant correlations with grain yield were a negative correlation with maturity and a positive correlation with boron tolerance, both of which are

consistent with the impact of a terminal drought/crown rot combination. The lines with early maturity would have been expected to perform much better under these circumstances, because they would have proceeded further through grain fill prior to severe moisture stress enhancing the crown rot infection.

Boron tolerance was most valuable under these conditions, as the roots of the tolerant lines would probably have grown further into the subsoil and had access to more water, reducing, or delaying plant death. This effect was observed in a field experiment conducted by (the late) Mr. Jeremy Dennis at Hart on a boron toxic subsoil in 2001 (unpublished data). The seed of durum lines included in the trial had been inoculated with *Fusarium pseudograminearum* to provide two treatment levels of the disease (plus and minus). During grain fill, many of the inoculated plots suffered significant plant death, but the boron tolerant lines appeared more resistant, as measured by grain yield, whereas growth room experiments conducted by the SARDI team led by Dennis had previously shown that these lines were no more resistant than other durum genotypes. The mean concentration of B in the whole tillers sampled at Port Pirie (see Chapter 5) was 20mg/kg, which is above the critical value of 15mg/kg (Reuter *et al.*, 1997).

The Redhill site failed to indicate any benefit of either boron tolerance or Na exclusion, despite having subsoil levels above those indicative of toxicity (Chapter 3, Table 3.1). Hence, the variation in maturity and other deleterious characters from the donor parent were the main factors affecting yield in the terminal drought. The change in maturity, from a score of three (equivalent to Kalka) to five accounted for a yield reduction of approximately 200g/plot, or 39%. This was reflected in the later maturing lines (score 5) having 14% screenings compared to 8% in the lines with equivalent maturity to Kalka.

This result suggests that the dominant subsoil factor affecting yield at Redhill was high pH, as was found in 2001 (Chapter 3, Section 3.2). While the subsoil did contain toxic levels of both boron and salt (Table 3.1), the plants were also affected by high pH, which restricted root growth and consequently reduced the uptake of subsoil moisture. Consequently, there was no yield benefit associated with either boron or salt tolerance. The 2001 field trial was mapped for all three subsoil constraints, but the trend in grain yield in the durum variety Tamaroi was only observed in response to increasing pH (Cooper, 2004).

The mean grain yield of the BC_3F_2 derived lines was 10% below that of Kalka at Redhill, due to undesirable introgressions of chromatin from the donor parent, Na49. In the absence of any benefit conferred by the Na exclusion gene, the yield of the Na excluding lines was only 93% of that of the non-excluding lines. These Na excluding lines carry an additional segment of donor chromatin around the Na-excluding locus itself, as well as a random distribution of donor genetic material throughout the genome. This linked segment of DNA will range in size between lines, on the basis of random recombination events and will only be reduced in size by further cycles of backcrossing and recombination. The variation in grain yield resulting from this foreign DNA could have contributed to the large amount of variation not explained by the known variables (Na exclusion, boron tolerance and maturity), which was shown to be correlated across sites (Table 7.4).

Fortunately, several Na-excluding lines out-yielded Kalka, indicating that deleterious genetic material is unlikely to be closely linked to the locus of interest. The four highest yielding lines (mean of Two Wells and Redhill) which possess both boron tolerance and Na exclusion have been multiplied for more extensive yield and quality testing in future

years in the hope of identifying lines for release, or parental use in crossing programs (Table 7.8).

Table 7.8. High yielding, boron tolerant, Na-excluding BC_3F_2 derived lines from the cross (Kalka#4*Na49) multiplied for extensive yield and quality testing. Grain yield expressed as a percentage of Kalka.

Line	Glasshouse Na (mg/kg)	Pt. Pirie Na (mg/kg)	B solution root length (mm)	Two Wells yield	Redhill yield	Mean yield (two sites)
93	810	465	125	124	99	112
95	710	1375	115	132	108	120
171	950	1125	132	118	102	110
192	830	395	138	106	99	103
Kalka	7050	9373	128	100	100	100

The Two Wells experiment was sown later than optimum due to the quarantine restrictions applied in response to the discovery of Wheat Streak Mosaic virus in the bird proof enclosure at the Waite Campus. Consequently, the drier than average September had a very large impact on yield and screenings, so boron tolerance and Na exclusion were both important in mitigating these conditions. The large screenings percentage was also reflected in the larger grain (>2mm) containing very little endosperm (Plate 7.1), greatly reducing its value in semolina milling. The combination of boron tolerance and Na exclusion resulted in a large increase in yield and a corresponding reduction in screenings, which emphasises the importance of combining tolerance to the subsoil constraints. A similar benefit of boron tolerance and Na exclusion may have been evident at Redhill if Kalka had been more tolerant of high pH.

The work described in this chapter is important, in that it represents the first major attempt to investigate the effect of Na exclusion on salt tolerance in the field in an equivalent genetic background. Further, the population developed has proved to be an invaluable tool for studying relationships between the various soil constraints.

Chapter 8.

THE EFFECT OF Na EXCLUSION ON THE UPTAKE OF OTHER ELEMENTS IN DURUM WHEAT

8.1 Introduction

The theory that reduced Na uptake will improve salt tolerance in the *Triticea* has resulted in the publication of many studies investigating the relationship between Na uptake and potassium. Many authors have concluded that Na exclusion mechanisms operate by K/Na discrimination, or the selective uptake of K in place of Na. None of these studies have investigated concentration change of other elements in response to Na exclusion.

One of the earliest published recordings of a connection between Na uptake and salt tolerance was that of Greenway and Rogers (1963). They found that a salt tolerant accession of *Agropyron elongatum* (tall wheat grass) had restricted transport of Na and Cl to the shoot, particularly the youngest leaf blades, maintaining high K/Na ratios. Following on from this discovery, Story *et al.* (1985) observed that when the *Agropyron elongatum/Triticum aestivum* amphiploid was grown in saline media it had a lower Na and higher K concentration in the shoot than the *Triticum aestivum* parent of the amphiploid, Chinese Spring.

Gorham *et al.* (1987) produced a set of D genome substitution lines in the background of the durum variety Langdon. They indicated that a gene or genes on the long arm of chromosome 4D controlled the enhanced Na exclusion and preferential uptake of K by hexaploid wheat compared to tetraploid durum and suggested that this operated by controlling the discrimination between sodium and potassium in uptake and transport to the

shoots. Gorham (1988) later demonstrated that the K/Na discrimination was related to loading of salts into the xylem. Dvorak *et al.* (1994) used homoeologous recombination to produce a series of 4D/4B (Chinese Spring/Langdon) recombinant lines and concluded the K/Na discrimination was due to a single co-dominant gene on 4DL, which was named *Kna1*. It can be observed in the data published by Gorham *et al.* (1987), Gorham (1988) and Dvorak *et al.* (1994) that the change in Na concentration attributed to the *Kna1* gene was never fully matched by a change in K concentration, with the change in K only between fifty and eighty-five percent of the change in Na. A similar discrepancy was noted by Dubcovski *et al.* (1996) in the data obtained while mapping the *Kna1* locus, but they did not conclude that the name *Kna1* was somewhat of a misnomer.

Munns *et al.* (2000) screened a wide range of tetraploid *Triticum turgidum* for low Na uptake and enhanced K/Na discrimination. This study identified three accessions from the subspecies *durum* that had low Na uptake and K/Na discrimination of a similar magnitude to *Triticum aestivum*. One of these was identified by Munns *et al.* (2000) as Na49, which was the donor parent used in the production of the population described in Chapter 5. Munns *et al.* (2000) concluded that the level of discrimination of the genetic mechanism in the durum Na49 was at least equivalent to the effect of *Kna1* and that it was possibly homoeologous to that locus in bread wheat.

Both the *Kna1* locus and the Na exclusion gene of Na49 have a very large effect on Na^+ concentration. This would affect the net charge, solute concentration and water relations if not balanced by changes in the concentration of other ions. If the plant achieved this by simply increasing or decreasing the uptake of other elements, the change in K concentration could be expected to be greatest due to its inherently high uptake from the

soil. The change in concentration of other elements relative to their actual concentration in the soil solution could be of the same magnitude, but balance a far smaller proportion of the change in Na.

8.2 Relationship between Na and the other elements in the genetic study reported in Chapter 5.

8.2.1 Introduction

The large population, described in Chapter 5, afforded a unique opportunity to investigate the effects of Na exclusion on the uptake of other elements, as the data obtained by ICP spectrometry provided simultaneous measurement of the concentration of fifteen other elements.

Most of the comparisons in this study have been made primarily on the basis of simple correlations, but in some experiments multiple regression analysis has also been used to elucidate information within the homogeneous high and low Na classes (i.e. variation in Na not related to the major Na exclusion gene). Many elements were correlated with Na concentration and interrelated within these groups. Where a complex set of inter-relationships occurs it is useful to determine which characters are most closely related to the genetic control of the trait of interest (Na) and the simplest method of identifying the components that account for that variation is through multiple regression analysis (Snape, 1987).

8.2.2 Materials and methods

The concentration of sixteen elements was simultaneously determined by ICP spectrometry in the experiments described in Chapter 5. This chapter primarily investigates the effect of Na concentration on the other elements measured in each experiment. Therefore, the design of each experiment is not described here, but can be referred to in Chapter 5. The ground plant samples from the Two Wells site were also tested for Cl concentration by PIRSA ACML at Loxton, SA, because Cl is not measured by the ICP spectrometer of Waite Analytical Services.

There was substantial variation in maturity among the BC_3F_2 derived lines at each site, ranging from equivalent to Kalka, to quite late maturing. The stage of development of the lines was scored at Port Pirie on the tenth of September and Redhill on the tenth of October on a scale of one to five, where one was very early and five, very late. The maturity of the recurrent parent Kalka was equivalent to a score of three. Unfortunately, the Two Wells site was not scored and consequently, the lines only grown at that location remained unscored.

Correlation coefficients have been calculated between Na concentration and the concentration of all detected elements using Microsoft Excel 2000. The multiple regression analysis was performed using Genstat edition 6 and was initially undertaken with the inclusion of all detected elements. The least significant variate was removed from the equation in each subsequent reiteration of the analysis, until all variates remaining were significant at the 5% level when subjected to a t-test. In this way, a simple equation that explained the maximum amount of variation in Na concentration was calculated.

Variation in the severity of salinity was mapped at the Port Pirie field site using an EM38 conductivity meter in the upright orientation (measuring conductivity to a depth of 1.5m) on each of the Kalka check plots. The conductivity of the plots located between the Kalka check plots was estimated from the nearest check plot on either side (within the bay) and weighted on the basis of distance. This appeared to be an appropriate estimate as most of the variation was between bays, with only slight variation within.

8.2.3 Results

The correlations between Na and all other elements detected by ICP-Spectrometry have been calculated from the data obtained in studying the genetic control of the Na exclusion trait of Na49 in Chapter 5 of this thesis. The relevant sections of Chapter 5 for each data set are tabulated in Table 8.1.

Table 8.1. The sources of data analysed in Chapter 8, Section 2 for populations segregating for the Na exclusion gene of Na49.

Data Set	Material	Chapter
A	BC_1F_1 derived lines grown at Roseworthy, 2000	5.2
B	$BC_1F_{1,3}$ derived single plants grown in saline pots	5.2
C	BC_3F_1 plants grown in saline pots	5.3
D	BC_3F_2 derived lines grown in saline pots	5.4
E	BC_3F_2 derived lines grown in plots at Port Pirie	5.5
F	BC_3F_2 derived lines grown in plots at Two Wells	5.5

Of the sixteen elements measured by ICP-Spectrometry, five were not detectable in any of the data sets (Mo, Co, Ni, Cd and Al), except data set E, were Al was detectable, but not

significantly correlated with Na. The correlations of the other elements (including Cl) with Na are presented in Table 8.2.

Table 8.2. Correlation coefficient (r) for the relationships between Na and all other detected elements measured in the whole tillers of populations segregating for the Na exclusion gene of the durum Na49, in the background of the variety Kalka.

Data Set		Elements										
		Fe	Mn	B	Cu	Zn	Ca	Mg	K	P	S	Cl
A	r	0.15	0.18	0.08	0.22	0.10	-0.21	-0.17	**-0.39**	0.02	0.01	
	Sig.	ns♣	ns	ns	ns	ns	ns	ns	**	ns	ns	
B	r	0.07	-0.06	-0.08	0.11	0.05	0.00	0.02	**-0.24**	0.01	0.07	
	Sig.	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	
C	r	-0.04	-0.17	0.16	-0.10	**-0.40**	-0.02	**-0.42**	**-0.65**	-0.15	0.05	
	Sig.	ns	ns	ns	ns	***	ns	***	***	ns	ns	
D	r	-0.11	0.01	0.06	-0.08	0.07	**-0.21**	**-0.48**	**-0.40**	0.01	0.06	
	Sig.	ns	ns	ns	ns	ns	*	***	***	ns	ns	
E	r	0.04	0.03	0.04	0.11	-0.05	**-0.50**	**-0.50**	**-0.62**	0.12	**0.25**	
	Sig.	ns	ns	ns	ns	ns	***	***	***	ns	**	
F	R	0.04	-0.03	0.05	0.07	0.00	-0.40	-0.07	**-0.85**	0.23	**0.83**	**0.73**
	Sig.	ns	ns	ns	ns	ns	ns	ns	***	ns	***	***

♣ not significant; * (P<0.05); ** (P<0.01); *** (P<0.001).

Potassium

The concentration of K increased in response to the decreased Na accumulation, associated with the Na exclusion gene of Na49, in all data sets (Figure 8.1). This is consistent with the K/Na discrimination mechanism of Na exclusion proposed by Munns *et al.* (2003). The increase in K concentration can be expressed as a percentage of the decrease in Na concentration from the slope of the line of best fit. These percentages are provided in Table 8.3, and indicate that in the five data sets, the change in K concentration accounts for between forty and fifty-one percent of the change in Na.

Data sets D, E and F detail the elemental composition of the BC_3F_2 derived lines from the cross (Kalka#4*Na49). These were segregating for the single Na exclusion gene of Na49, with three groups of genotypes visible in Figure 8.1, parts (d), (e) and (f) and in Figures 8.3, 8.4 and 8.5. To make these genetic groups more clearly discernable to the reader, Figure 8.1 (e) is presented in an alternative way in Figure 8.2. The homogeneous non-excluding lines (derived from homozygous high Na F_2 plants) are presented in blue, the heterogeneous lines (derived from heterozygous F_2 plants) are presented in pink and the homogeneous excluders (derived from homozygous low Na plants), in black.

Figure 8.1. The relationship between Na and K concentration (mmol/kg) in whole tillers in five populations (data sets) segregating for the Na exclusion gene of Na49, in the genetic background of the durum variety Kalka.

(a) Data set A, BC_1F_1 derived lines grown at Roseworthy, 2000,

(b) Data set B, $BC_1F_{1,3}$ derived single plants grown in saline pots,

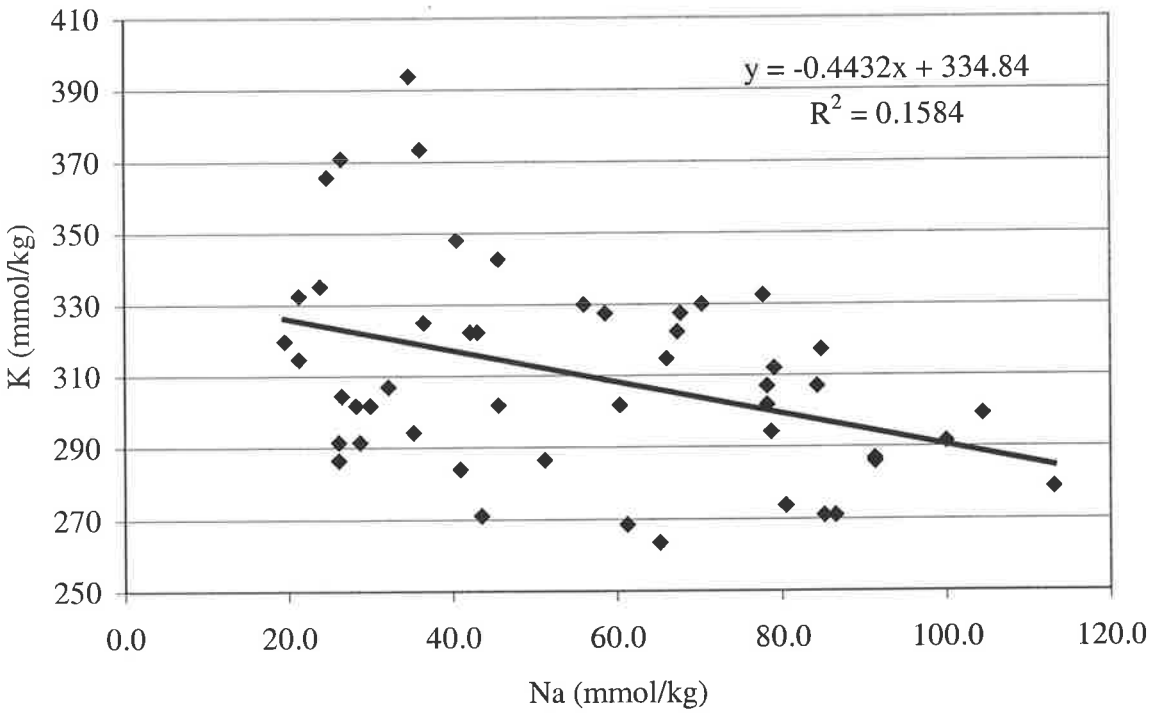
(c) Data set C, BC_3F_1 plants grown in saline pots,

(d) Data set D, BC_3F_2 derived lines grown in saline pots,

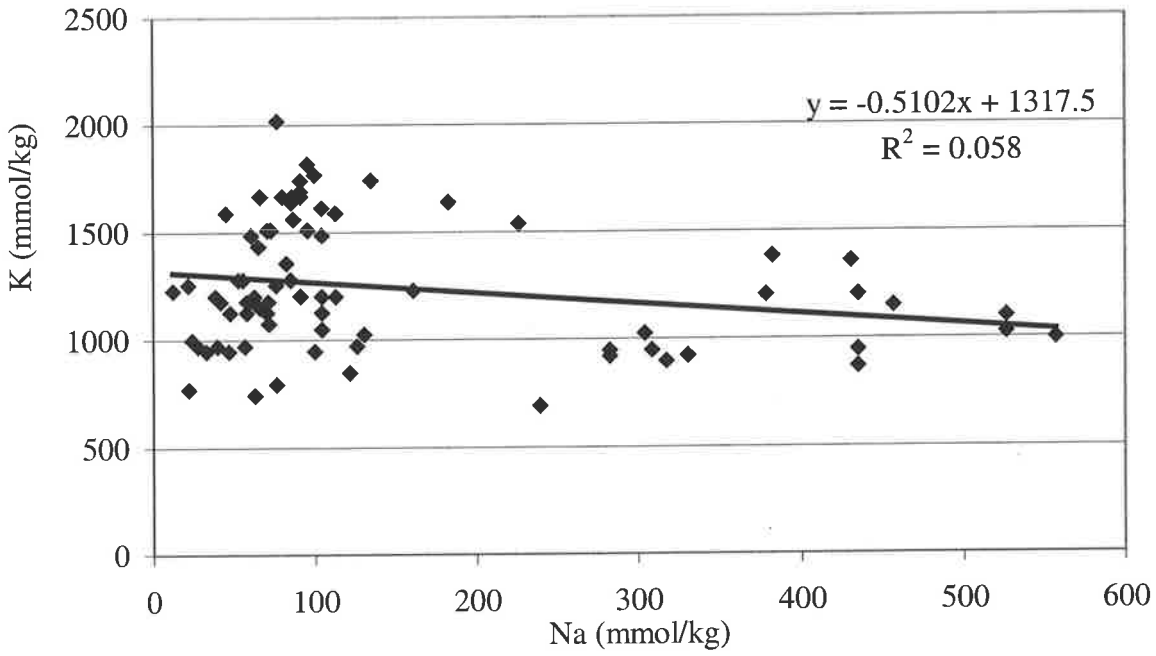
(e) Data set E, BC_3F_2 derived lines grown in plots at Port Pirie,

(f) Data set F, BC_3F_2 derived lines grown in plots at Two Wells.

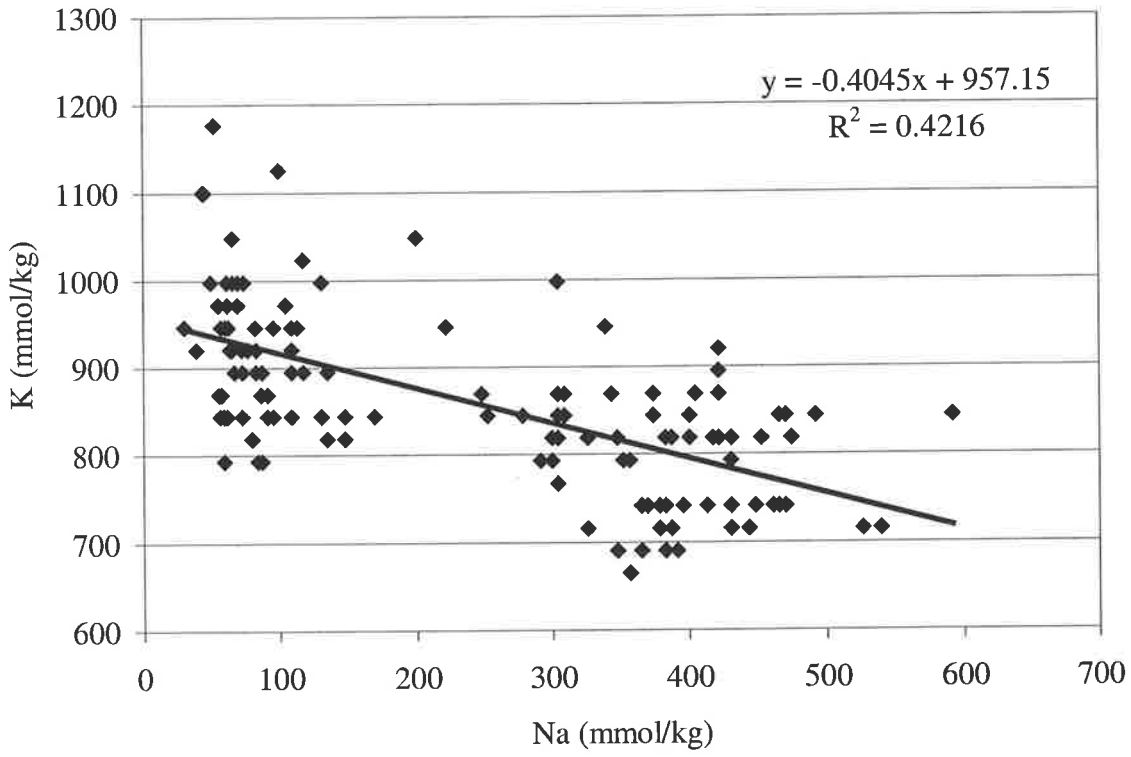
(a) Data set A, forty-eight BC_1F_1 derived lines grown at Roseworthy, 2000.



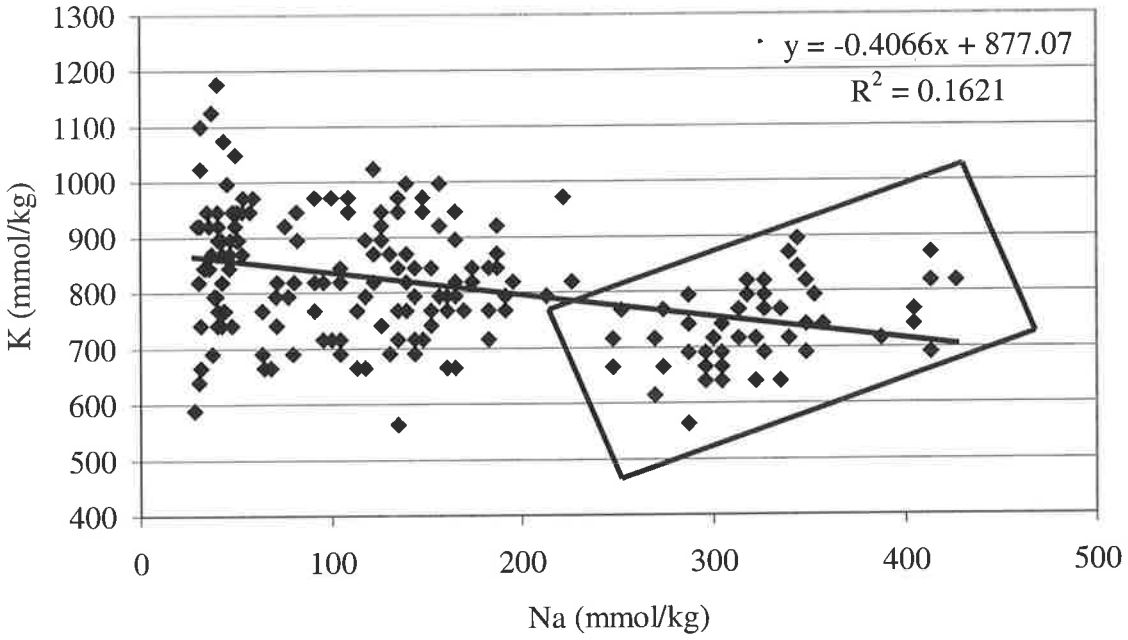
(b) Data set B, seventy-eight $BC_1F_{1,3}$ derived single plants grown in saline pots.



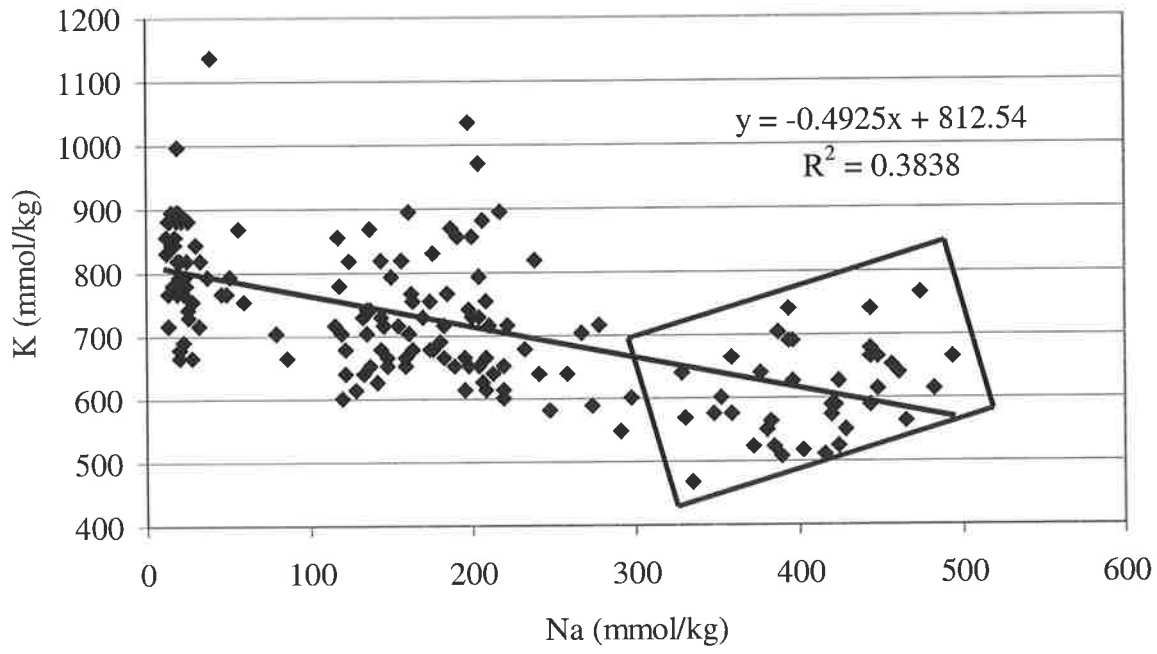
(c) Data set C, 126 BC_3F_1 plants grown in saline pots.



(d) Data set D, 193 BC_3F_2 derived lines grown in saline pots (homozygous non-excluding lines enclosed in box).



(e) Data set E, 164 BC_3F_2 derived lines grown in plots at Port Pirie (homozygous non-excluding lines enclosed in box).



(f) Data set F, twenty-two BC_3F_2 derived lines grown in plots at Two Wells.

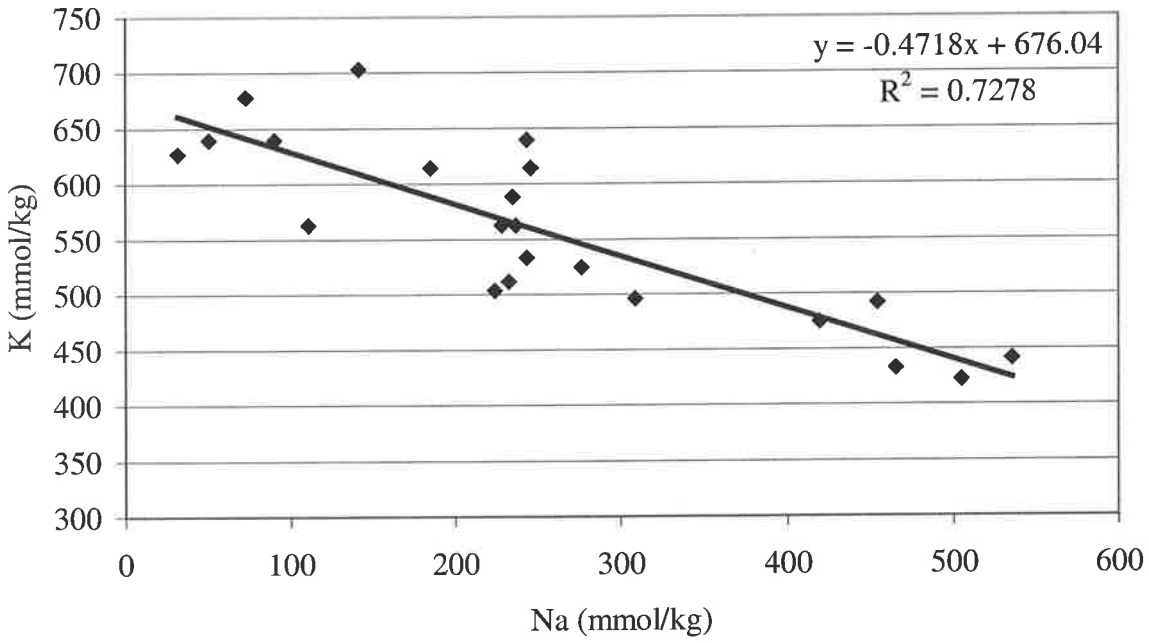
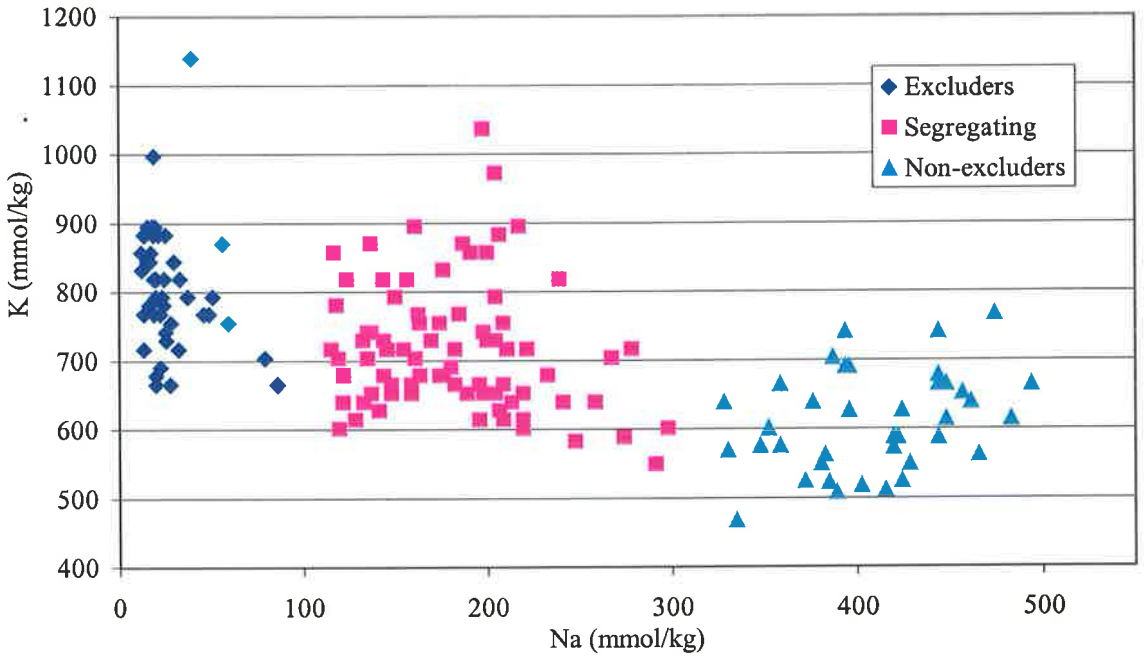


Table 8.3. The percentage of the change in Na accounted for by the change in K in ICP-spectrometry data sets from populations segregating for the Na exclusion gene of Na49.

Data set	Slope	Change in K as a % of change in Na
A	-0.443	44.3
B	-0.510	51.0
C	-0.405	40.5
D	-0.407	40.7
E	-0.493	49.3
F	-0.472	47.2

Figure 8.2. The relationship between Na and K concentration (mmol/kg) in the whole tops of 164 BC_3F_2 derived F_4 lines grown in field plots at Port Pirie.



Magnesium

The concentration of Mg increased in response to Na exclusion in data sets C, D and E (Figure 8.3) and was expressed as a percentage of the decrease in Na concentration from the slope of the line of best fit (Table 8.4). The change in Mg concentration accounted for between 1.3 and 3.0 percent of the change in Na in the data sets where a significant correlation was detected (C, D, and E).

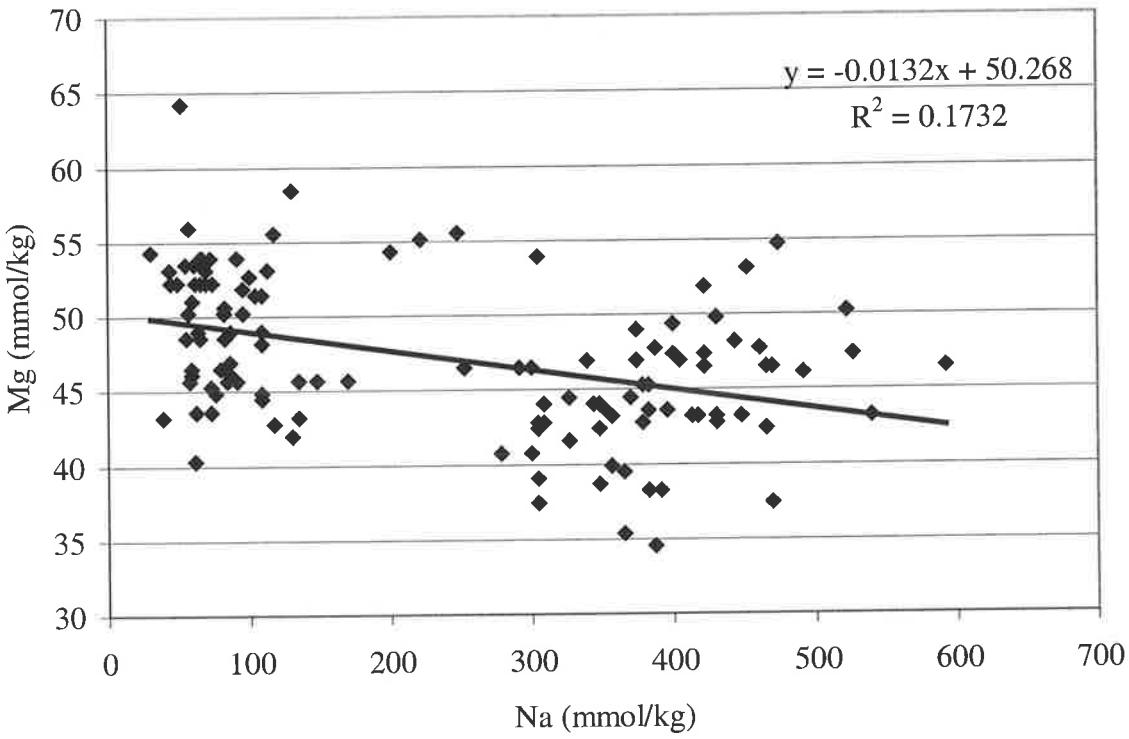
Figure 8.3. The relationship between Na and Mg concentration (mmol/kg) in whole tillers in three populations (data sets) segregating for the Na exclusion gene of Na49, in the genetic background of the durum variety Kalka.

(a) Data set C, BC_3F_1 plants grown in saline pots,

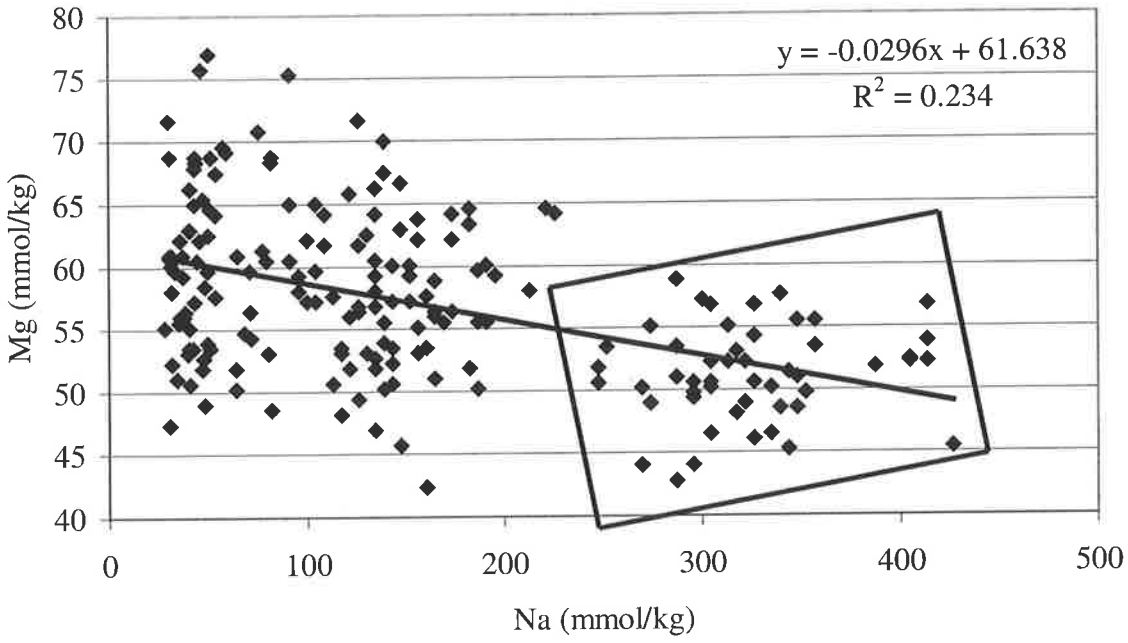
(b) Data set D, BC_3F_2 derived lines grown in saline pots,

(c) Data set E, BC_3F_2 derived lines grown in plots in the paddock at Port Pirie.

(a) Data set C, 126 BC_3F_1 plants grown in saline pots.



(b) Data set D, 193 BC_3F_2 derived lines grown in saline pots (homozygous non-excluding lines enclosed in box).



(c) Data set E, 164 BC_3F_2 derived lines grown in plots at Port Pirie (homozygous non-excluding lines enclosed in box).

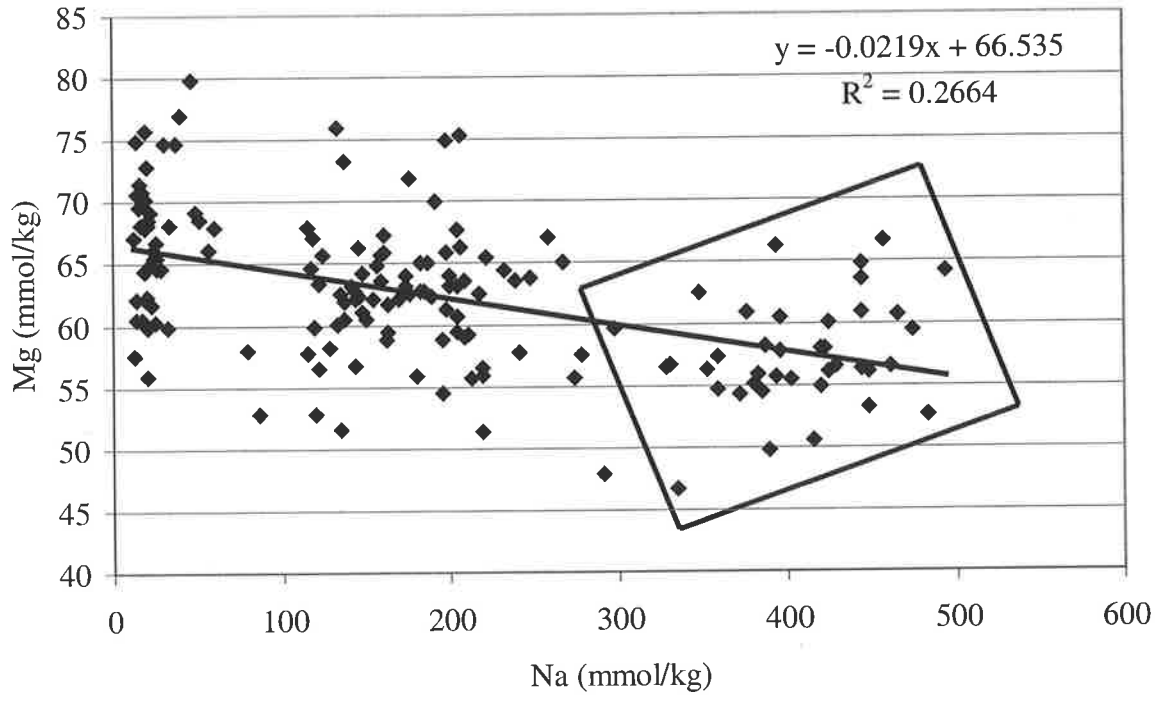


Table 8.4. The percentage of the change in Na accounted for by the change in Mg in ICP-spectrometry data sets from three populations segregating for the Na exclusion gene of Na49.

Data set	Slope	Change in Mg as a % of change in Na
C	-0.0132	1.3
D	-0.0296	3.0
E	-0.0219	2.2

Calcium

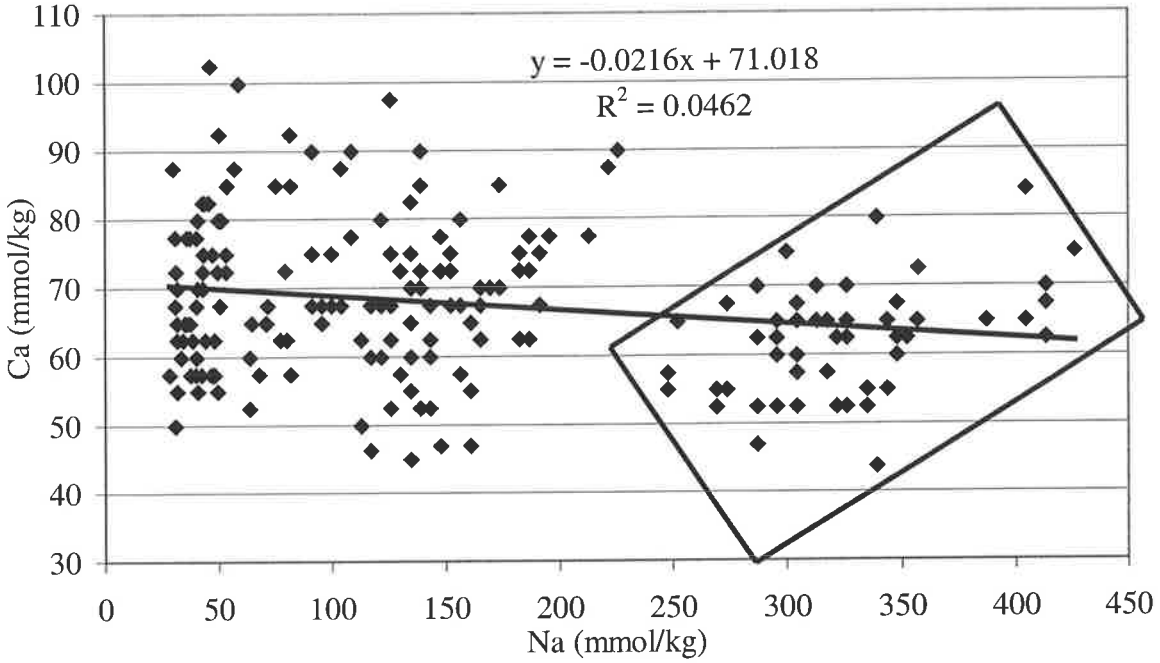
Significant negative correlations between Ca concentration and Na in whole tillers were detected in two of the data sets (Table 8.2). This was consistent with the other two macronutrient cations, K and Mg, in having increased uptake in response to the reduced Na uptake of lines containing the Na exclusion gene of Na49 (Figure 8.4). These relationships resulted in the change in Ca concentration accounting for 2.2 to 2.6 percent of the change in Na concentration (Table 8.5).

Table 8.5. The percentage of the change in Na accounted for by the change in Ca in ICP-spectrometry data sets from two populations segregating for the Na exclusion gene of Na49.

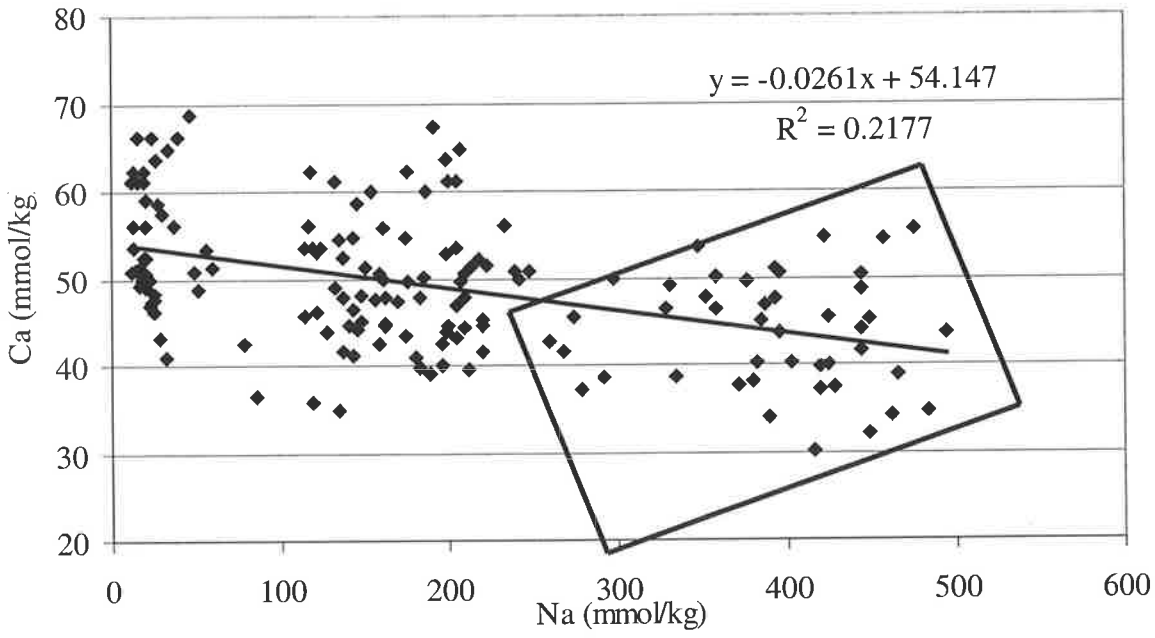
Data set	Slope	Change in Ca as a % of change in Na
D	-0.0216	2.2
E	-0.0261	2.6

Figure 8.4. The relationship between Na and Ca concentration (mmol/kg) in whole tillers in two populations (data sets) segregating for the Na exclusion gene of Na49, in the genetic background of Kalka.

(a) Data set D, 193 BC_3F_2 derived lines grown in saline pots (homozygous non-excluding lines enclosed in box).



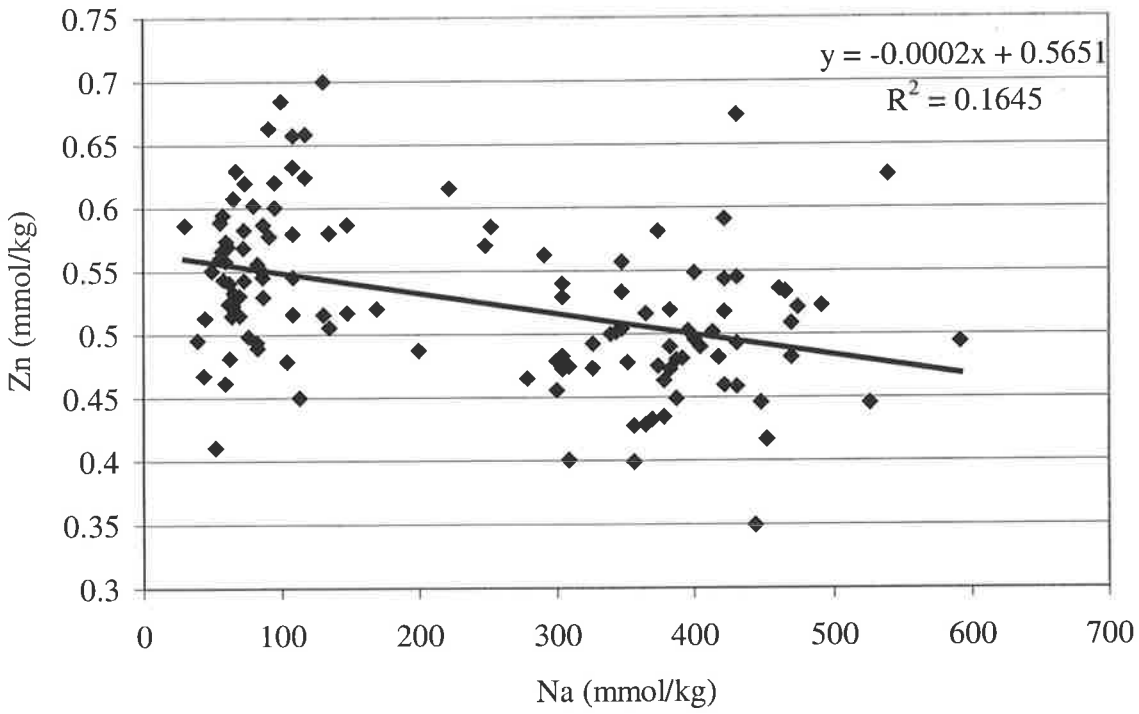
(b) Data set E, 164 BC_3F_2 derived lines grown in plots at Port Pirie (homozygous non-excluding lines enclosed in box).



Zinc

Zinc was significantly correlated with the uptake of Na only once (Table 8.2). This, as with the other cations, was a negative correlation (Figure 8.5). The change in zinc concentration only accounted for 0.02 percent of the change in Na concentration in response to the presence of the Na exclusion allele of Na49.

Figure 8.5. The relationship between Na and Zn concentration (mmol/kg), in the primary tillers of 126 F_1BC_1 plants (data set C) grown in saline pots in a glasshouse.

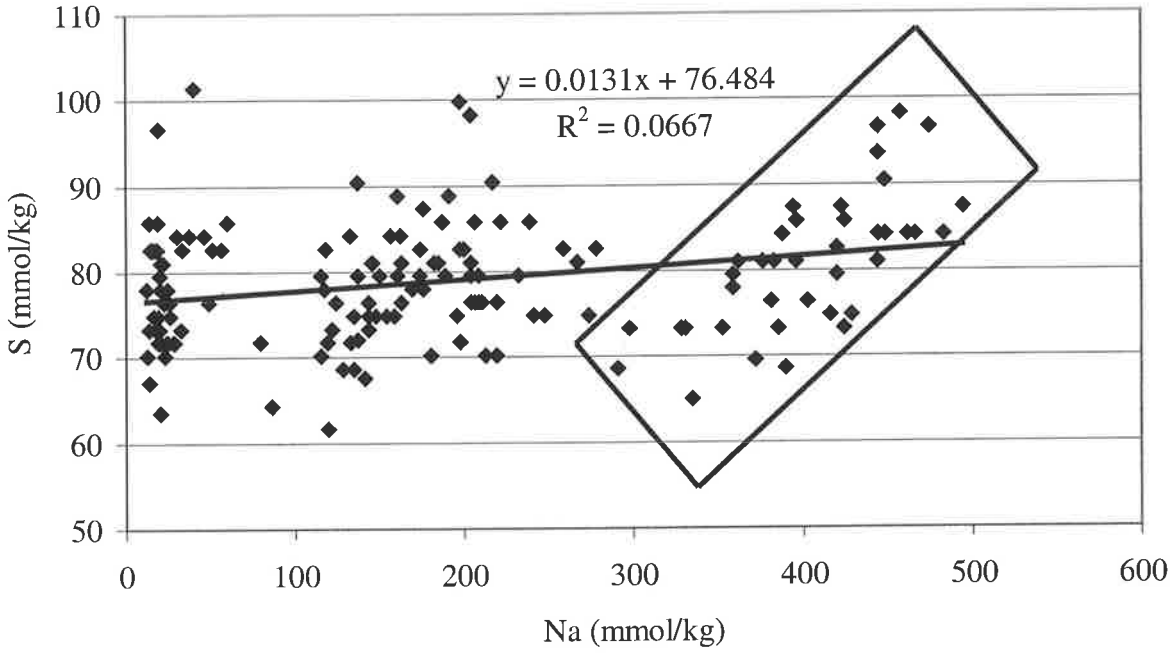


Sulphur

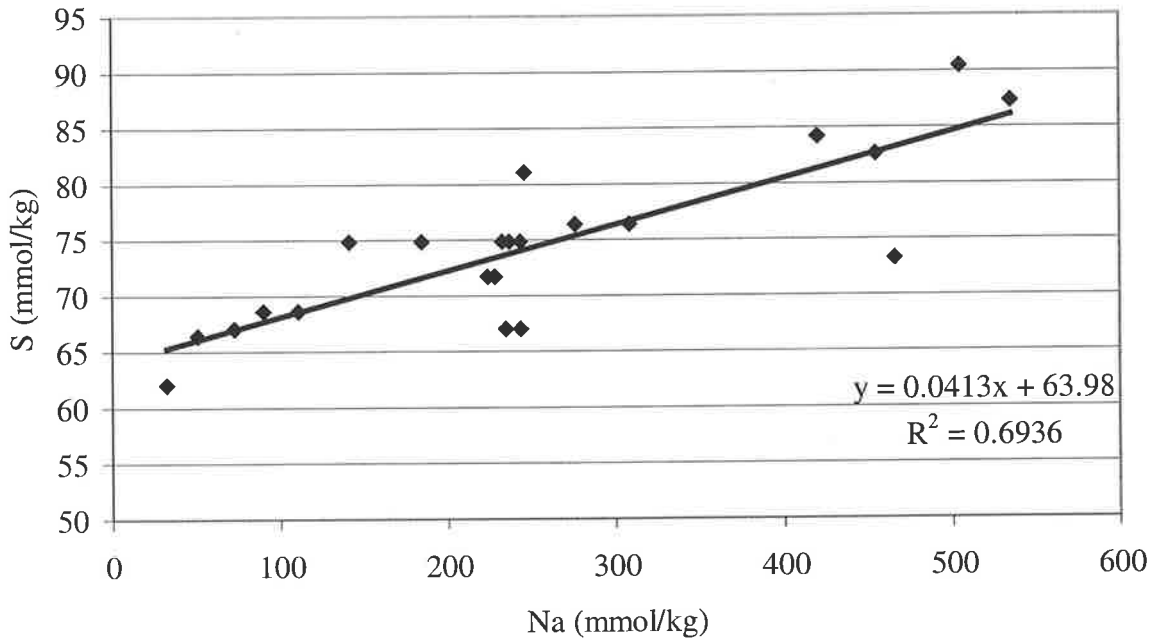
Correlations between S and Na were significant in two of the data sets (Table 8.2). Unlike the cations, the accumulation of S was positively correlated with Na; that is, the Na excluding lines contained lower concentrations of S (Figure 8.6). The slope of the line of best fit (Figure 8.6) indicated that the change in S concentration was equivalent to 1.3 and 4.1 percent of the change in Na in the two data sets.

Figure 8.6. The relationship between Na and S concentration (mmol/kg) in whole tillers in two populations (data sets) segregating for the Na exclusion gene of Na49, in the genetic background of the durum variety Kalka (homozygous non-excluding lines enclosed in box).

(a) Data set E, 164 BC_3F_2 derived lines grown in plots at Port Pirie,



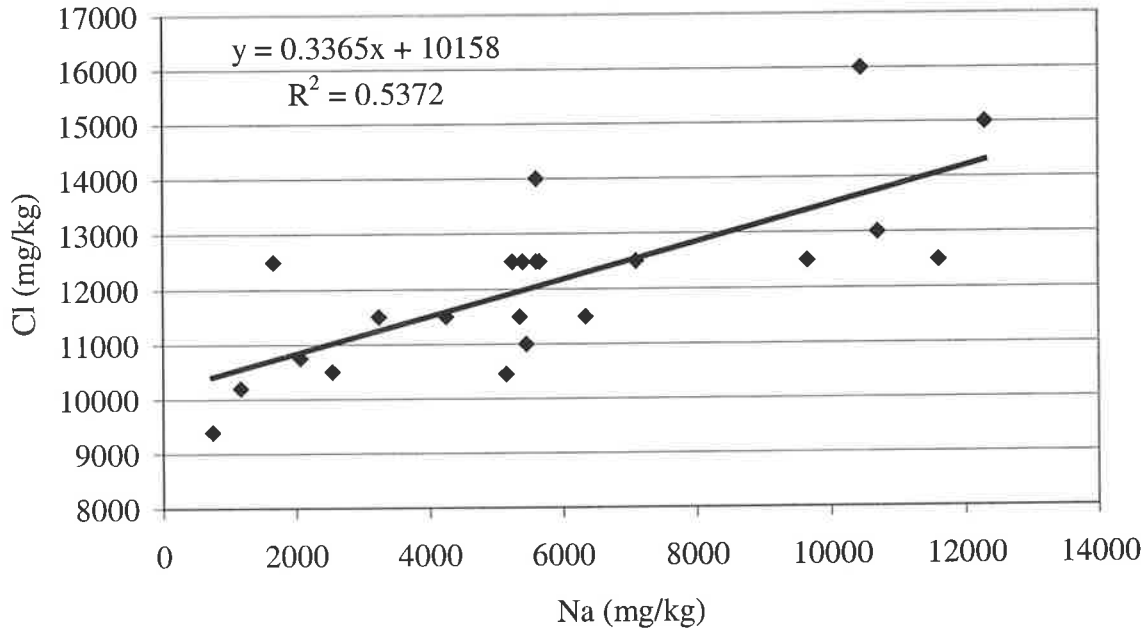
(b) Data set F, twenty-two BC_3F_2 derived lines grown in plots at Two Wells.



Chloride

Chloride concentration was significantly correlated ($P<0.001$) with Na at Two Wells; the only data set analysed. Like S, the anion Cl^- was positively correlated with Na, indicating that the Na excluding lines also had lower Cl accumulation (Figure 8.7). This change in Cl concentration was equivalent to 33.7% of the change in Na, which is second in magnitude only to K.

Figure 8.7. The relationship between Na and Cl concentration (mmol/kg) in whole tillers of a population of twenty-two BC_3F_2 derived lines segregating for the Na exclusion gene of Na49, in the genetic background of the durum variety Kalka. Tillers sampled from field plots grown at Two Wells, 2003.



It is clear that the change in K concentration is the major contributor to ionic balancing brought about by the reduced Na concentration of the Na excluding lines (40-51 percent), followed by Cl (33.7%) in the only data set in which it was measured. While the other elements described (Mg, Ca, Zn and S) do not account for a large percentage of the change

in Na, the change in their concentration in response to Na exclusion was not disproportionate when considered as a percentage of their concentration in non-excluding plants (Table 8.6).

Table 8.6. The change in other elements in response to Na exclusion in populations segregating for the Na exclusion gene of Na49, in the background of the variety Kalka.

Element	Change in element as a % of change in Na	Change in element as a % of concentration in non-excluders
K	40.5 – 50.0	10 – 44
Mg	1.3 – 3.0	11 – 15
Ca	2.2 – 2.6	11 – 25
Zn	0.02	12
S	1.3 – 4.1	-5 – -25
Cl	33.7	-29

Correlations with Na within the homogeneous high Na group, in data sets D and E.

Figure 8.2 depicts the relationship between Na and K concentration in the whole tillers of the lines sampled at Port Pirie, with the groups separated into the three Na exclusion genotypes by the use of different icons. The homogeneous high Na lines have also been enclosed within boxes in Figures 8.1, 8.3, 8.4 and 8.6, to highlight the observation that the distribution of individuals around the line of best fit, within the high Na group does not appear to be 'normal' in any of these relationships. That is, within the high Na group, the lines with lower Na concentration contain lower than expected concentrations of the other elements, while the lines with higher Na concentration contain higher concentrations of

other elements. The correlations between Na and all other detected elements in these two data sets have been calculated to further investigate this observation (Table 8.7).

Table 8.7. Correlation coefficients (r) within the Na excluding lines for the relationships between Na concentration and the concentrations of elements detected in the whole tillers sampled from a glasshouse experiment (data set D) and from the Port Pirie field site (data set E), for the BC_3F_2 derived population.

Data set		Element									
		Fe	Mn	B	Cu	Zn	Ca	Mg	K	P	S
D	r	0.05	**0.43**	0.12	-0.21	-0.06	**0.44**	**0.39**	**0.44**	0.10	**0.31**
	Sig.	ns♣	***	ns	ns	ns	***	***	***	ns	*
E	r	**0.67**	0.00	0.11	**0.76**	**0.42**	-0.20	0.31	0.33	**0.79**	**0.65**
	Sig.	***	ns	ns	***	**	ns	ns	ns	***	***

♣ not significant; * (P<0.05); ** (P<0.01); *** (P<0.001).

Within the high Na accumulating group of lines, the correlation coefficients between Na and all other elements that were statistically significant were positive (Table 8.7). This indicated that the factors affecting the accumulation of Na were also affecting the accumulation of all elements taken up from the soil. The factors likely to contribute to this variation were variation in growth rate, soil nutrient concentration, rooting density, or nutrient uptake efficiency. The correlation coefficient of 0.22 for the relationship between the Na concentration in whole tillers in the pot experiment (data set D) and the Na concentration at Port Pirie (data set E) was close to, but not statistically significant. This suggested that most of the variation in Na concentration was due to factors which differed

in each experiment and did not arise from segregation of an additional minor gene controlling Na exclusion.

The effects of spatial variation in transient salinity and maturity on nutrient uptake at Port Pirie

The correlations between transient salinity as measured by the EM38 conductivity meter, maturity and all elements measured in the plant tops are presented in Table 8.8.

Significant correlations were observed between soil salinity and seven of the twelve elements detected. As expected, the correlation with tissue Na was highly significant, while the accumulation of Ca was negatively correlated, which was probably due to the ionic balance mechanism. If this was the case, it could be surprising that K and Mg were not also negatively correlated with salinity except that areas with high Na levels probably also had high K and Mg levels. Significant correlations were also recorded with Fe (+ve), Mn (-ve), Cu (+ve) and Al (+ve).

Maturity was highly correlated ($P<0.001$) with an increase in all elements except Mn, B, Ca, Mg and Al. There were no significant negative correlations. This indicated that one of the main factors causing variation in Na and other elements within this homozygous Na excluding group was maturity.

Table 8.8. Correlation coefficients (r) between salinity (measured by EM38 conductivity survey to a soil depth of 1.5m) and maturity score and concentration of 12 elements measured in the plant tops by ICP-spectrometry and sampled from 38 homogeneous high Na accumulating BC_3

F_2 derived F_4 lines grown at Port Pirie (data set E).

	Elements detected by ICP spectrometry											
	Fe	Mn	B	Cu	Zn	Ca	Mg	Na	K	P	S	Al
Salinity	0.44	-0.45	0.04	0.39	0.11	-0.60	-0.10	0.63	-0.17	0.54	0.16	0.72
	**	**	ns	*	ns	***	ns	***	ns	***	ns	***
Maturity score	0.78	0.23	0.19	0.52	0.50	0.27	0.23	0.50	0.78	0.65	0.70	0.14
	***	ns	ns	***	***	ns	ns	***	***	***	***	ns

ns (not significant); * (P<0.05); ** (P<0.01); *** (P<0.001).

Variation in Na concentration within the homogeneous non-excluding lines

The variation in Na concentration among the homozygous non-excluding lines was correlated with salinity, maturity, and many other elements (Table 8.8). The data were analysed using multiple linear regression to elucidate which environmental factors (salinity and the concentration of other elements in soil) affected Na accumulation. Maturity score was also included in the analysis, because of its significant correlation with Na concentration. To maximise the amount of environmental variation included in the analysis, regression was undertaken on all 76 plots (2 replicates of each line), rather than the 38 line means. The equation explaining the greatest percentage of variation in Na concentration is described in Table 8.9.

Table 8.9. Parameter estimates of the equation best describing variation in Na concentration in whole tillers sampled from 38 homogeneous high Na BC_3F_2 derived lines of the cross (Kalka#4*Na49). Tillers were sampled from field plots at Port Pirie (data set E).

	Estimate	s.e.	t (68 df.)	t pr.
Constant	-573	1045	-0.55	0.585
Al	-63.9	22.5	-2.84	0.006
B	39.8	11.8	3.36	0.001
Salinity	13.33	4.42	3.02	0.004
K	-0.1936	0.0437	-4.43	<0.001
Mn	-45.7	14.2	-3.23	0.002
P	1.087	0.345	3.15	0.002
S	4.270	0.489	8.73	<0.001

These parameters explained 82.0% of the variation observed in Na concentration in the high Na lines. As expected, salinity had a significant effect on Na concentration. Conversely, the effect of maturity time was not sufficiently large enough to be retained in the equation.

The elements K, Mn, P and S were included in the equation, but were also highly correlated with maturity (Table 8.8). It is possible that these elements were a surrogate for maturity at this site, through being a more accurate prediction of the actual maturity differences than the observed maturity score. Multiple regression analysis was conducted to test which of the variates K, Mn, P and S were acting as surrogates for maturity. This analysis revealed that 57.7% of the variation in maturity was accounted for by K and Mn concentration alone.

The effects of high Na accumulation on plant nutrition

The results of the experiments described in this chapter have identified significant correlations between Na exclusion and the accumulation of several other elements (Table 8.10). It is clear from this summary that the concentration of other cations increased in lines possessing the Na exclusion trait, while the concentrations of the anions SO_4^{2-} and Cl^- decreased.

Table 8.10. Concentration of elements (mg/kg) correlated with Na concentration in the whole tillers of high and low Na lines selected from populations segregating for the Na exclusion gene of Na49. Correlations presented in Table 8.2.

Data Set	Element	Correlation with Na	Range[A] (mg/kg)	Mean of non-excluders[C]	Mean of Na excluders[B]
A Roseworthy	K	-0.39**	10300-15400	11890	12473
B Pots	K	-0.24*	27000-79000	41500	50683
C Pots	K	-0.65***	26000-46000	31432	36070
C	Mg	-0.42***	841-1560	1093	1200
C	Zn	-0.40***	22.9-45.8	32.5	36.4
D Pots	K	-0.40***	22000-46000	28872	34283
D	Mg	-0.48***	1030-1870	1260	1445
D	Ca	-0.21*	1750-4000	2507	2743
E Port Pirie	K	-0.62***	17000-45000	22400	31852
E	Mg	-0.50***	1135-1940	1398	1614
E	S	0.25**	1975-3250	2613	2493
E	Ca	-0.5***	1210-2750	1898	2154
F Two Wells	K	-0.85***	15900-29000	19100	25250
F	S	0.83***	1860-3000	2625	2105
F	Cl	0.73***	9400-16000	13800	10670

* ($P<0.05$); ** ($P<0.01$); *** ($P<0.001$); [A] Range in concentration of all observations;

[B] Mean concentration in Na excluding lines; [C] Mean concentration in non-excluding lines.

Some of the observations for individual cations, summarised in Table 8.10, have values greater than the critical values published for deficiency; however there are some exceptions. The critical values for Mg concentration in whole shoots at Feekes Scale (FS)

10 (equivalent to Zadocks 40-60) range from 1300-1500mg/kg (Reuter *et al.,* 1997). On this basis the Mg concentrations measured in whole shoots sampled from both of the pot experiments (data sets C and D) are marginal to deficient (Table 8.10), as are a number of the field plots grown at Port Pirie (data set E), where the Na excluding group had a mean Mg concentration of 1614mg/kg, only marginally above the upper level of the critical value of 1500 mg/kg (Reuter *et al.,* 1997).

Calcium was marginal to deficient in the field plots at Port Pirie. Reuter *et al.* (1997) reported a critical value of 2500 mg/kg, with deficiency symptoms observed at 2000 mg/kg. The mean Ca concentration in the whole shoot of the high Na lines was 1898 mg/kg, but while the mean of the low Na lines was higher (2154 mg/kg), it was still below the critical value of 2500 mg/kg. Deficiency symptoms were not observed, but the concentration of Ca in the plants was significantly correlated ($P<0.001$) with both yield and screenings (Figure 8.8). Calcium concentrations below 1600mg/kg restricted grain yields to less than 50g/plot (Approx. only the seeding rate), while above this concentration, yields were highly variable and ranged from 0 to 230g/plot.

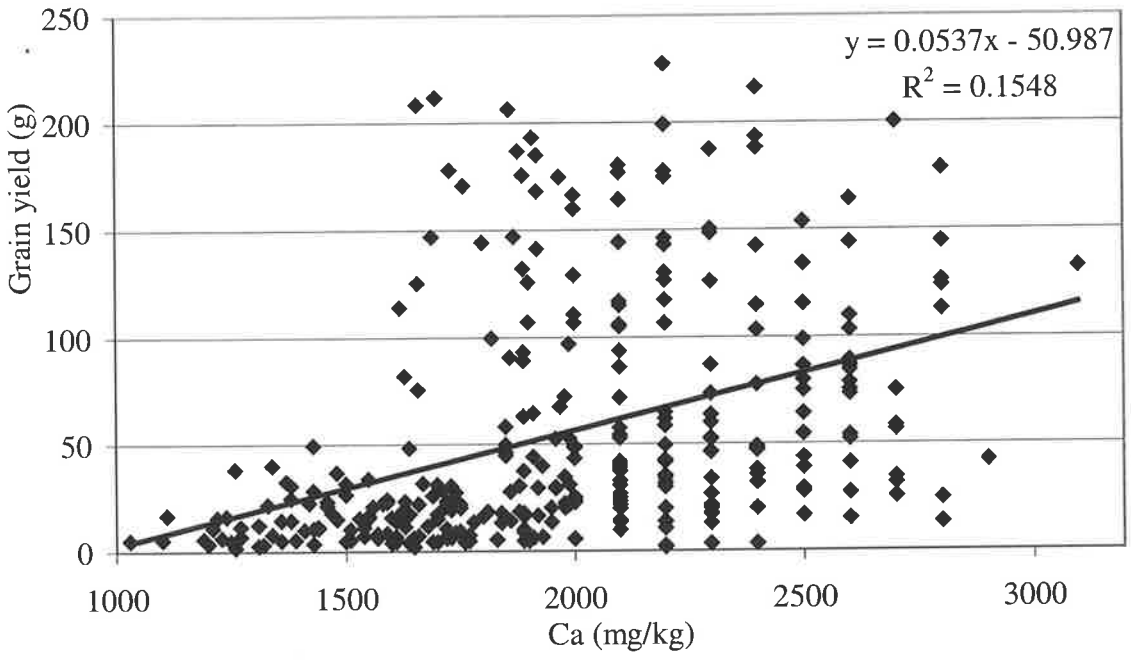
Reuter *et al.* (1997) quoted a critical value of 18000 mg/kg of K in whole tops at FS10, with two recordings of deficiency symptoms occurring below 15000 mg/kg. While the K concentrations in whole tops sampled from the two pot experiments were above the critical value, the field plots ranged from marginal to deficient. The K concentration in whole tops at Roseworthy (data set A) showed that, while the low Na lines had a higher mean K concentration than the high Na lines, they were also below the critical value of 18000 mg/kg. While, the mean value for the high Na lines sample at Port Pirie (data set E) was 22400 mg/kg (above the critical value), some individual high Na plots did have K

concentrations below the critical value, but this was not reflected in correlations with yield, or screenings.

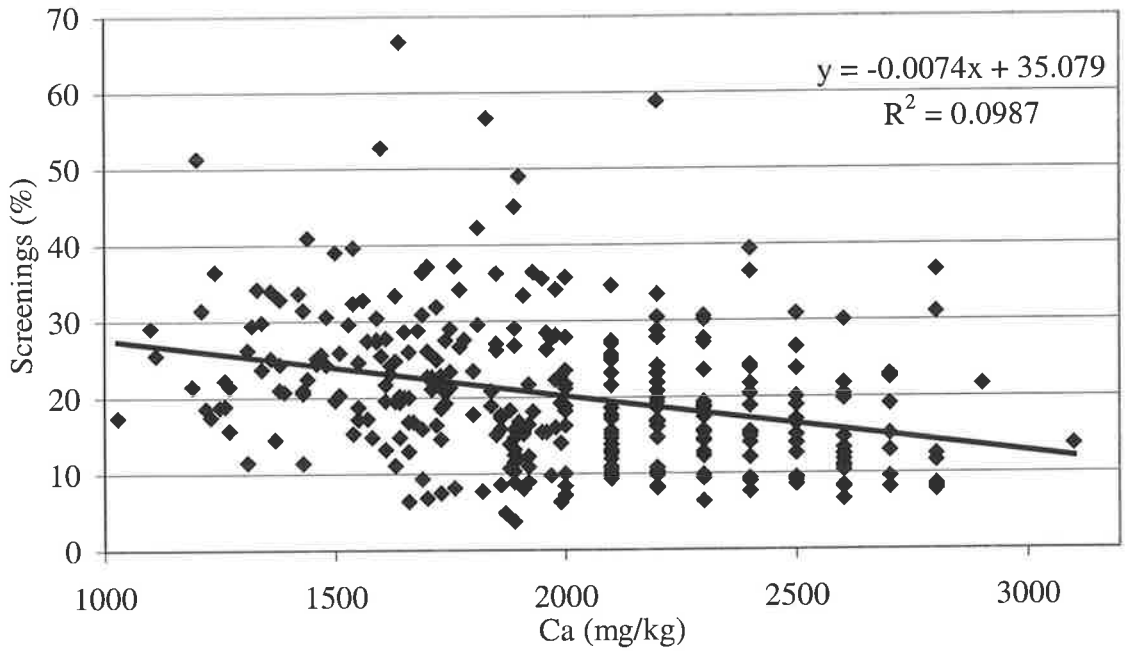
Many of the plots of high Na lines sampled at Two Wells had K concentrations between the marginal and critical values. Significant correlations ($P<0.001$) were detected between K and both yield and screenings. Because of the highly significant effect of Na on yield and screenings (Tables 7.2 and 7.6), multiple linear regression was conducted, including both K and Na as the explanatory variates. It was found that only Na was retained in the equations best describing yield and screenings, suggesting that the correlations between these and K only occurred because of the correlation between Na and K.

Figure 8.8. The relationship between plant Ca concentration (mg/kg) and (a) yield; and (b) screenings; in two replicates each of 164 BC_3F_2 derived lines from the cross (Kalka#4*Na49) grown at Port Pirie, 2003.

(a) The relationship between plant Ca (mg/kg) and grain yield (g/plot).



(b) The relationship between plant Ca (mg/kg) and screenings (%).



8.2.4 Discussion

The results of this investigation proved that Na exclusion did not result specifically from Na/K discrimination, and that this theory has become incorrectly accepted. The fact that the change in K never accounted in full for the change in Na is clear in the results published by Gorham *et al.* (1987), Gorham, (1988) and Dvorak *et al.* (1994), but all of these authors ignored the evidence.

Na is excluded from the above ground parts of the plant by either a root exclusion, or efflux mechanism, which in the absence of an ionic adjustment would cause a charge imbalance. This imbalance is at least partially offset by the adjustment of other ions, so that the uptake of other cations was increased in Na excluding lines, while the uptake of anions was reduced.

The difference in accumulation of the cations Ca, K and Mg, between Na-excluders and non-excluders was shown in several instances to be the difference between adequate nutrition and deficiency at field sites affected by transient salinity.

Calcium deficiency symptoms were not observed at the Port Pirie site despite the marginal concentration measured in the whole shoots; however a highly significant correlations were detected between plant Ca and both grain yield and screening (Figure 8.8). Ca deficiency had previously been observed in Tamaroi field plots on a saline site at Jamestown, whereas neighbouring bread wheat plots were symptomless (see Chapter 3). ICP analysis of whole tillers of the forty-eight Tamaroi check plots in the Jamestown experiment revealed that the Ca concentration ranged from 1270 to 2900 mg/kg, straddling the critical value of 2500 mg/kg (Reuter *et al.,* 1997). The Port Pirie concentrations were in a similar range to those measured at Jamestown and it is likely that other environmental factors, including crown rot, were probably responsible for masking the expression of symptoms at Port Pirie.

The concentrations indicating K deficiency, measured in the Roseworthy samples (2000), and the marginal concentrations measured at Port Pirie and Two Wells in 2003, could be explanations for some of the inferior grain yields of durum relative to bread wheat in South Australia. The established, successful durum growing areas are characterised by subsoil salinity levels below 4dS/m ECe (see Chapter 2), whereas much of the area where durums have not been grown have subsoils above 4dS/m. The uptake of potassium would be greater from non-saline soils than from subsoils with soil conductivity levels above 4dS/m, unless the increase in soil Na was matched by an increase in K. While the aim of this project is to improve the salt tolerance of durum wheat by reducing the uptake of Na, it is

equally possible that a benefit of the low Na accumulation may arise from an alleviation of K deficiency on the soils where transient salinity occurs.

The effects of K deficiency in a terminal drought are very similar to those of salt toxicity (Marschner, 1988). Plants affected by water stress have higher K requirements, primarily because of the importance of maintaining high stomatal K concentrations. The plants also need high K concentrations in the vacuoles to ensure that high tissue water content is maintained. Under K deficient conditions plants translocate K from the mature stems and leaves, which under severe deficiency become chlorotic and necrotic, reducing the photosynthetic area and thereby the assimilate available to fill grain. Abscisic acid levels increase in the grain in response to low K, resulting in a shortened grain fill period and lighter single grain weights (Marschner 1988). Under these conditions, the situation could be exacerbated if the only water available to the plant is subsoil moisture. In soils affected by transient salinity, the uptake of Na with subsoil moisture would be higher and have an even greater competitive effect on the uptake of K.

The negative relationship between Zn and Na uptake which occurred in the pot experiment (experiment B) was notable, as it has been observed previously that durum wheats are less Zn and Mn efficient than bread wheats (Cakmak *et al.*, 1996b; Graham, 1988; Kaur *et al.*, 1989; Lewis, *et al.*, 2001; Saberi, *et al.*, 2001). These elements are taken up as divalent cations and, like the macronutrient cations, may have higher rates of accumulation in Na excluding plants.

The decrease in the uptake of the anions SO_4^{2-} and Cl^- in response to Na exclusion could possibly result in S deficiency in Na excluding genotypes; however, this is no more likely

than it would be in bread wheat, which excludes at least an equivalent quantity of Na. The more interesting relationship was the reduction in Cl uptake in response to Na exclusion. The effect of Cl toxicity on saline sites has not been investigated in detail in cereal crops, but it is known to be of importance in other crops such as grapes (Downton, 1977; Gibberd, *et al.* 2001). If chloride toxicity does occur in durum crops grown on saline sites, another indirect benefit of Na exclusion would be the reduction in Cl uptake.

Variation in Na concentration was observed within each of the two Na uptake genotypes of the BC_3F_2 derived population grown at Port Pirie (Figure 5.7). This variation is most clearly observed in the homozygous non-excluding lines. A large portion of the Na variation within the high Na class appears to be environmental in origin or due to genetic differences that are not directly related to the Na accumulation locus. One such genetically controlled difference was the maturity score, which was significantly correlated ($P<0.001$) with Na uptake at the Port Pirie site (Table 8.8), but was not included in the equation of best fit determined by multiple linear regression (Table 8.9). As the maturity scores used in the multiple linear regression were the mean of observations on a single occasion at Redhill and Port Pirie, it is possible that the mineral element concentrations that were positively correlated with maturity (Mn, B and Al) may have been a more accurate predictor of the maturity differences than the visual score, simply acting as a surrogate for a more accurate maturity score in the regression analysis.

8.3 Solution culture experiment to investigate the effect of Na exclusion on micronutrient uptake

8.3.1 Introduction

Durum wheat has been reported to have less efficient uptake of manganese (Graham, 1988; Kaur *et al.*, 1989; Saberi, *et al.*, 2001), zinc (Cakmak *et al.*, 1996; Lewis, *et al.*, 2001), copper and iron (Rathjen pers. comm.). Saberi (1999) concluded that the fact that durum wheats have been employed as inefficient check genotypes in micronutrient studies is evidence of the inefficient nature of the current durum cultivars. The observation that Zn uptake increased in response to Na exclusion in the BC_1F_1 plants grown in saline pots (Figure 8.5), suggested that the uptake of Zn and other cationic micronutrients may be greater in Na excluding genotypes.

The failure to detect other significant correlations between Na and the other trace elements could be the result of variation in micronutrient concentration in the substrate, or variation in other environmental factors affecting uptake of the minerals. To overcome this, a solution culture experiment was undertaken in a controlled environment, with the aid and advice of Dr. Yusef Genc. This experiment studied the relationship between the uptake of other elements and Na in a population of fifteen Na excluding and fifteen non-excluding lines.

8.3.2 Materials and methods

Apparatus and Conditions

Each line was represented in each replicate by a single cup (total of 32 entries, 30 lines and 1 cup each of Kalka and Na49). The plants were grown in 25mm diameter plastic cups (two plants/cup) with plastic mesh bottoms, which were fitted into 27mm holes in a 5mm PVC sheet. This apparatus was suspended over a 10L tank so that the bottom of the cups were 1-2mm above the solution. Aeration was provided by continuous flow of solution from the tank into a 3L sump containing a submersible electric pump, which continually circulated the solution to an inlet hole in the tank on the opposite side to the outlet. The tray volume was kept constant at 9L by the fixed height of the inlet and outlet. There were four separate apparatus, such that the experiment was a completely randomise design in four blocks (Plate 8.1).

The experiment was conducted in a growth room set at a ten hour, 15^{o}C night and fourteen hour, 20^{o}C day.

Plate 8.1. Hydroponic apparatus used to investigate the effect of Na exclusion on the uptake of other elements in Na excluding and non-excluding lines derived from the cross (Kalka#4*Na49).

Seed

Fifteen high and fifteen low Na BC_3F_2 derived lines from the cross (Kalka#4*Na49) were grown in comparisons with the parental lines Kalka and Na49. The BC_3F_2 derived lines were selected at random from the high and low Na peaks in the distribution of Figure 5.5, with high Na lines (non-excluders) from a range of 7500 – 9800 mg/kg, while the low Na group had a range of 650 – 860 mg/kg. Two of the putative low Na lines and one of the high Na lines were later shown to be misclassified segregating lines after analysis of the whole tops from the Port Pirie field trial, which resulted in the experiment actually comprising of thirteen homogeneous Na excluding lines, three segregating and fourteen homogeneous non-excluding lines.

Twenty seeds of each line, were surface sterilised using 2% sodium hypochlorite for ten minutes, rinsed and pre-germinated on moist filter paper in Petri dishes at 4°C for seven days. When the roots extended from all seeds by at least 10mm, two seeds were transplanted to each of the plastic planter cups and covered with 20mm of plastic beads to support the plants and reduce evaporation.

Solution

Twelve litres of nutrient solution was prepared for each individual apparatus by the method used by Genc (2004), except for the addition of 150mM NaCl and 10mM $CaCl_2$. Solutions were changed every seven days after transplanting (DAT) to ensure that adequate nutrient levels were maintained. The addition of NaCl and $CaCl_2$ began seven DAT. To reduce the osmotic shock, the salt concentration was increased by 50mM NaCl and 3.33mM $CaCl_2$ at seven, nine and eleven DAT. This was achieved by adding 100mL of 0.5M NaCl and

13mL of 0.5M $CaCl_2$ at each incremental increase in concentration. Evaporation loss was counteracted by the addition of double deionised H_2O when required.

Harvest and data collection

The plants were harvested twenty-seven DAT, when salt toxicity symptoms where observed and growth appeared to have slowed. The growth stage of the plants at the time of harvest ranged from fully tillered to early stem elongation. At harvest, fresh weights of plant tops and roots were recorded and these together with the youngest fully expanded blades (YEB) were sampled, dried for twenty-four hours at 70°C and analysed separately. The dry weight of plant tops and roots were recorded. The YEB and whole shoot samples where chopped finely using stainless steel scissors, digested using nitric acid and analysed for elemental composition by Waite Analytical Services, using ICP spectrometry.

8.3.3 Results

Plant growth

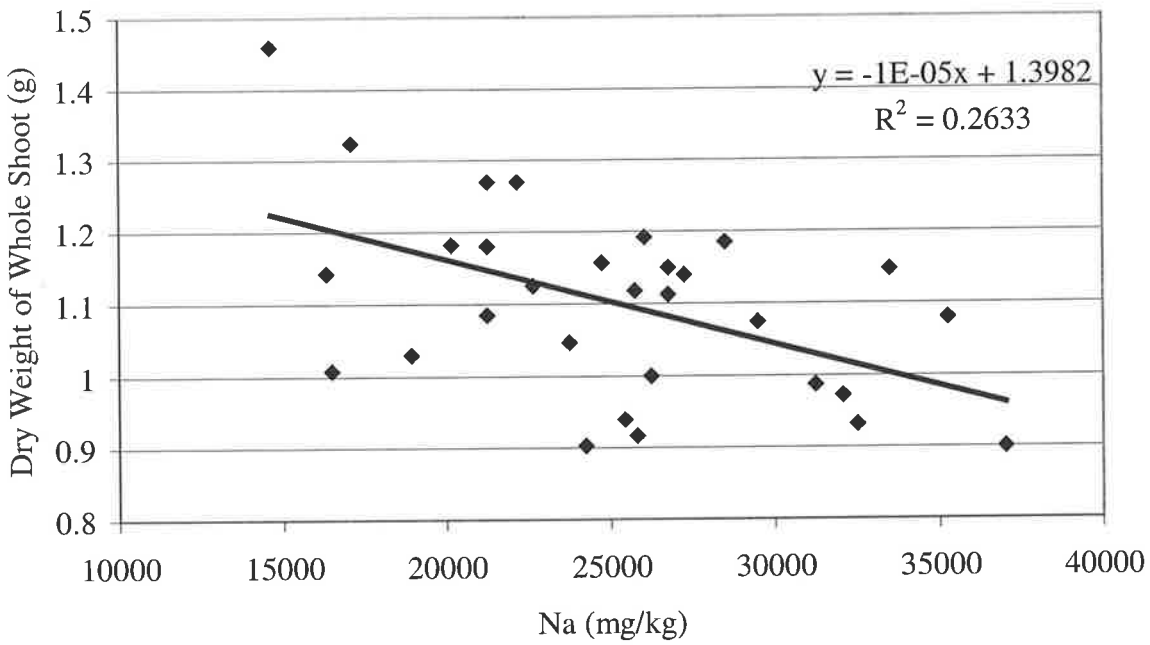
Separate analyses of variance were carried out for the parental lines and for the BC_3F_2 derived progeny.

Analysis of variance of the parental lines Kalka and Na49 revealed that the variates fresh shoot weight ($P<0.01$), fresh root weight ($P<0.01$), dry shoot weight ($P<0.05$) and dry root weight ($P<0.05$) were all significantly higher in the Na excluding parent, Na49. This was largely irrelevant, as the vastly different genetic background of Na49 may have contributed to the difference in biomass production, rather than any salt tolerance conferred by the Na exclusion locus.

Analysis of variance of the thirty BC_3F_2 lines revealed that the variates fresh shoot weight ($P<0.01$), dry shoot weight ($P<0.05$) and dry root weight ($P<0.05$) were all significant, while fresh root weight was not significant. Of the significant variates, dry shoot weight ($P<0.01$) and fresh shoot weight ($P<0.01$) were significantly negatively correlated with whole plant Na concentration (Figure 8.9), while only dry shoot weight was significantly negatively correlated with Na concentration in the YEB ($P<0.05$) (Figure 8.10).

Figure 8.9. The relationship between Na concentration in whole shoots of 30 BC_3F_2 derived lines and (a) dry weight; or (b) fresh weight of whole shoots grown in saline hydroponic solution for 27 days.

(a) Relationship between Na concentration in whole shoot and whole shoot dry weight.



(b) Relationship between Na concentration in whole shoot and whole shoot fresh weight.

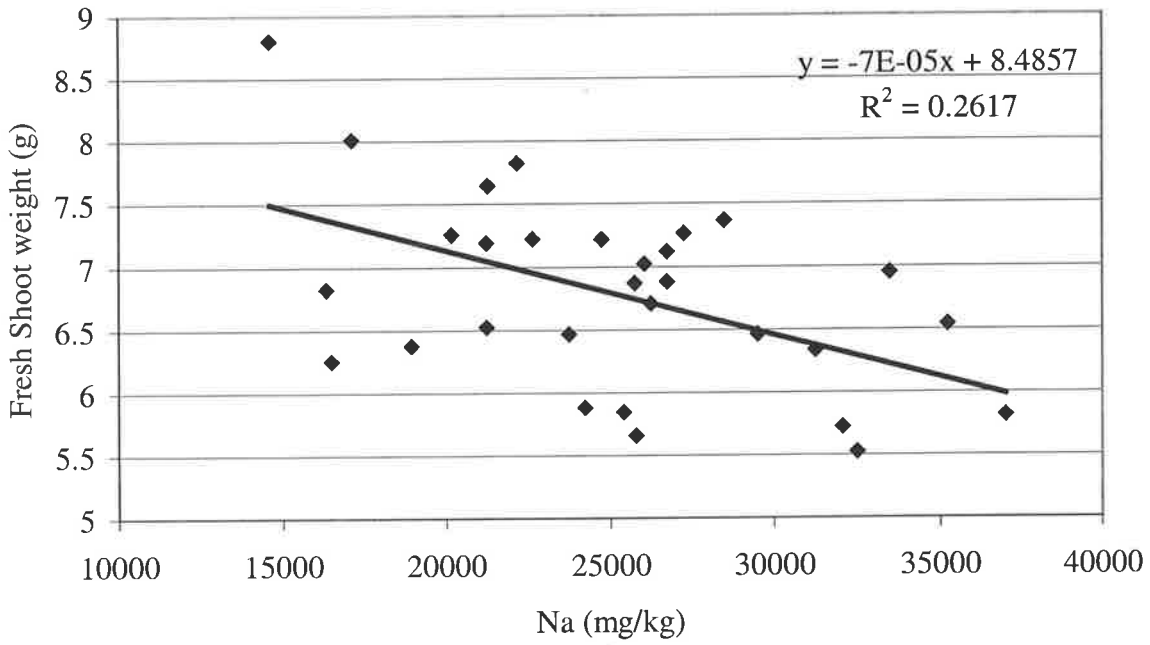
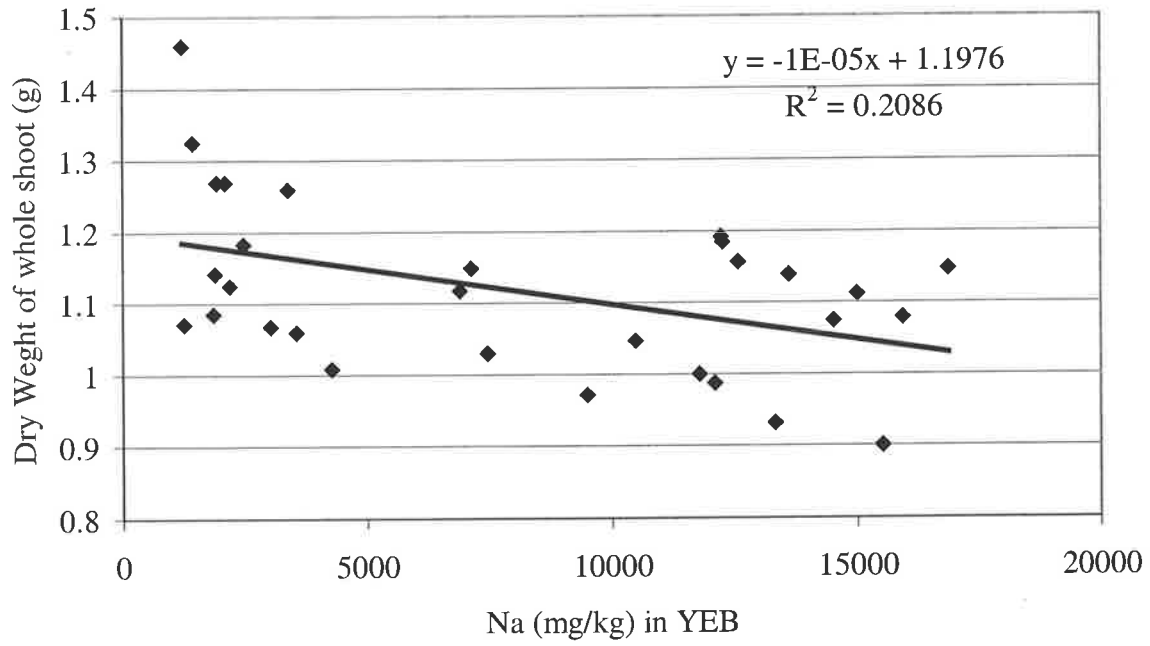


Figure 8.10. The relationship between Na concentration in YEB of 30 BC_3F_2 derived lines and dry weight of whole shoots grown in saline hydroponic solution for 27 days.

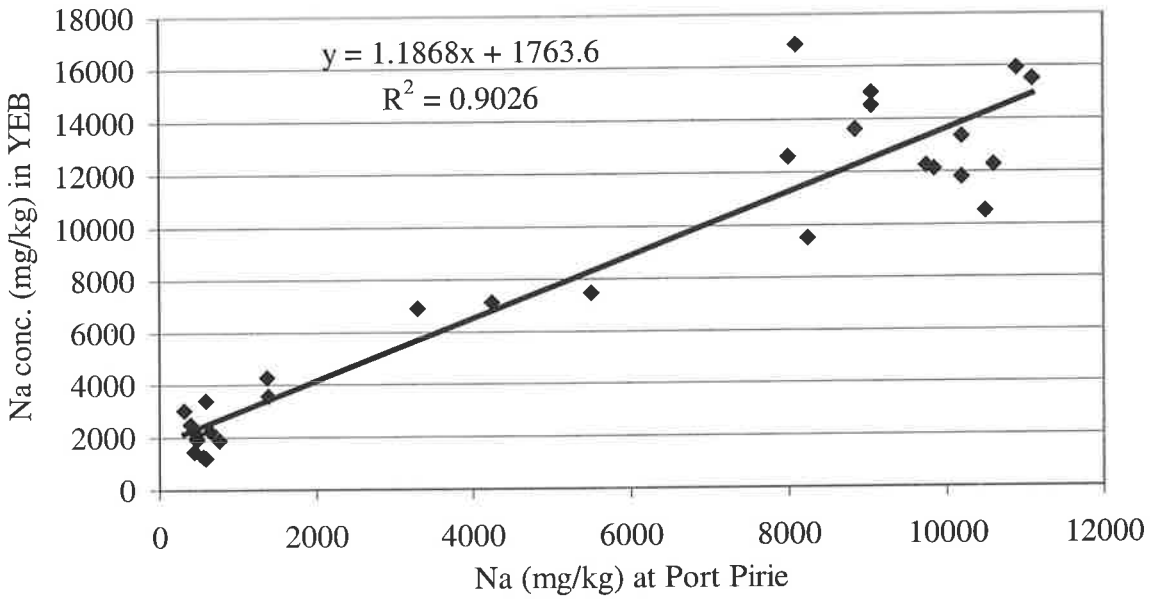


The relationship between Na concentration in the YEB and dry shoot weight is weak, but the effect of the Na exclusion gene can be seen, as the low Na lines can be distinguished from the three segregating lines and the high Na lines.

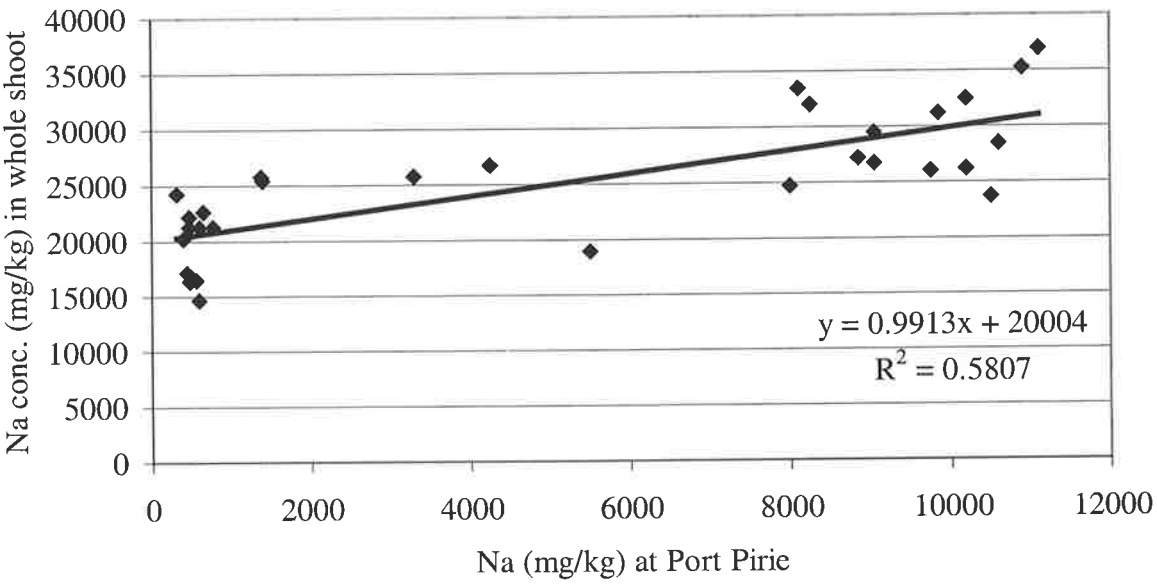
The relationship between whole shoot Na and both dry and fresh shoot weight was much stronger, however the effect of the Na exclusion gene on Na concentration in the whole shoot was much less obvious than it was in the YEBs. This was clearly illustrated by the comparisons of Na concentration in the whole tillers grown at Port Pirie with the Na concentrations measured in this solution culture experiment (Figure 8.11(b)), which clearly separated the lines into the three genotypic groups; homogeneous Na excluders (13), heterogeneous (3) and homogeneous non-excluders (14). The Na concentration in the YEBs (Figure 8.11 (a)), separated the lines into the same groups, while crossover occured between the groups for Na concentration on a whole shoot basis (Figure 8.11 (b)). Clearly, other factors affecting Na accumulation in the whole shoot also affected shoot weight, which resulted in the strong relationship between whole shoot Na concentration and whole shoot weight.

Figure 8.11. The relationship between Na concentration in the whole tillers of 30 BC_3F_2 derived lines grown at Port Pirie, with those of the same genotypes grown in solution culture.

(a) Comparison of Na concentration in whole tillers grown at Port Pirie, with Na concentration in the YEB of the same genotypes grown in solution culture.



(b) Comparison of Na concentration in whole tillers grown at Port Pirie, with Na concentration in the whole shoot of the same genotypes grown in solution culture.



Nutrient concentration in whole shoots

Of the sixteen elements measured by ICP spectrometry, twelve were at detectable levels. Analysis of variance of these elements in the whole shoots of the parental genotypes, Kalka and Na49, showed that Cu, Fe, Mn, Mo, P and Zn had significantly different concentrations in the whole shoots of the two genotypes. There was no significant difference in Na concentration between the two genotypes despite the highly significant differences measured in the pot experiments (see Chapter 5).

Analysis of variance of the elemental concentrations in the whole shoots of the thirty BC_3F_2 derived lines was performed independently of the parental data and this revealed that all twelve elements were significantly affected by genotype. Correlation coefficients were calculated with Na concentration in the whole shoot (Table 8.11).

Table 8.11. Correlation coefficient (r) for the relationships between Na concentration and eleven other elements measured in the whole tops of 30 BC_3F_2 derived F_3 lines. Plants grown in saline hydroponic solution for 27 days.

	Element										
	B	Ca	Cu	Fe	K	Mg	Mn	Mo	P	S	Zn
r	0.34	0.22	0.52	0.57	-0.70	-0.47	0.51	-0.15	0.62	0.25	0.63
Sig.	ns	ns	**	***	***	*	**	ns	***	ns	***
R^2			0.27	0.33	0.49	0.22	0.26		0.38		0.40

ns, not significant; *P<0.05; **P<0.01; ***P<0.001

Apart from the macronutrients K and Mg, all other significant correlations were positive, suggesting a dilution effect in faster growing, Na excluding plants; however, the

correlations between Na content (total uptake, rather than concentration) and the content of the other elements were very similar to the concentration correlations (Table 8.12). This suggests that it was likely that the uptake of the micronutrients Fe, Zn and Cu did not respond to Na exclusion in the same way as the macronutrients and that the positive correlation with Na concentrations was not due to a dilution effect in faster growing Na excluding plants.

Table 8.12. Correlation coefficient (r) for the relationships between Na content (mg/plant) and eleven other elements measured in the whole tops of 30 BC_3F_2 derived F_3 lines. Plants grown in saline hydroponic solution for 27 days.

	Element										
	B	Ca	Cu	Fe	K	Mg	Mn	Mo	P	S	Zn
r	0.18	0.09	0.40	0.38	-0.33	-0.18	0.38	-0.29	0.41	0.14	0.46
Sig.	ns	ns	*	*	ns	ns	*	ns	*	ns	*
R^2			0.16	0.14			0.14		0.17		0.21

ns, not significant; *P<0.05; **P<0.01; ***P<0.001

Nutrient accumulation in the youngest expanded blade

Analysis of variance on the concentration of elements in the YEBs of the parental lines Kalka and Na49, revealed that the concentrations of K, Mn, Na, P and Zn were all significantly affected by genotype. The Na excluding genotype, Na49, did have lower Na concentration in the YEB than Kalka, in contrast to the whole shoot concentration.

The analysis of variance of the elemental concentrations in the YEBs of the thirty BC_3F_2 derived lines showed that B, Fe, Mn and P were the only variates not significantly affected

by genotype. Correlation coefficients were calculated between the elements where there were significant differences and Na concentration in the YEB (Table 8.13).

Table 8.13. Correlation coefficient (r) for the relationships in the YEBs of 30 BC_3F_2 derived F_3 lines between Na concentration and the other six elements which had significant genotype effects. Plants grown in saline hydroponic solution for 27 days.

	Element					
	Ca	Cu	K	Mg	S	Zn
R	-0.343	0.57	-0.826	-0.747	0.591	0.828
Significance	ns	***	***	***	***	***
R^2		0.33	0.68	0.57	0.35	0.69

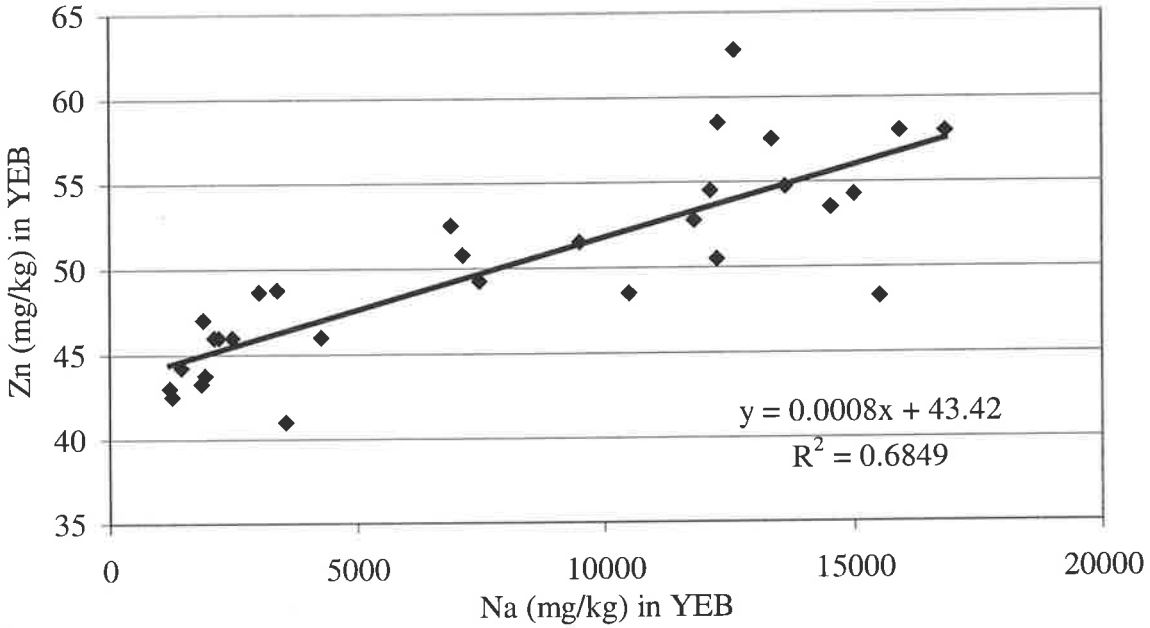
ns, not significant; *P<0.05; **P<0.01; ***P<0.001

The correlation between Na and the other macronutrient cations K and Mg, are negative, while a positive correlation occurred with the anion (SO_4^{2-}). This is consistent with the correlations observed in the pot and field experiments and summarised in Table 8.10. As with the correlations between elemental concentrations in the whole shoot, the micronutrients Cu and Zn were significantly positively correlated with Na (Figure 8.13), again suggesting that uptake of these cations was not enhanced by Na exclusion as it was with the macronutrients.

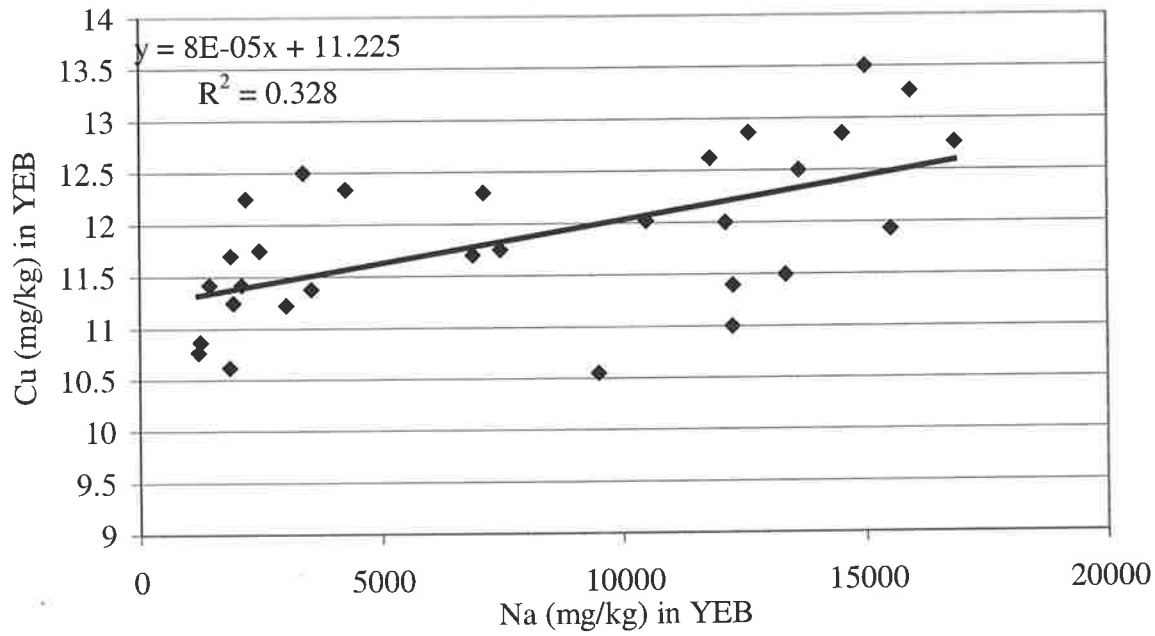
The correlations between elemental contents in the YEB could not be calculated because the dry weight of the YEB was not measured.

Figure 8.12. The relationship between Na concentration in the YEB of 30 BC_3F_2 derived lines and: (a) the concentration of Zn, and (b) the concentration of Cu. Plants grown in saline hydroponic solution for 27 days.

(a) The relationship between Na and Zn in the YEB of BC_3F_2 plants.



(b) The relationship between Na and Cu in the YEB of BC_3F_2 plants.



8.3.4 Discussion

The Na concentration in the whole tillers from Port Pirie, was more closely correlated with the Na concentration in the YEBs of plants grown in solution culture than it was with the Na concentration in the whole shoot (0.95 compared with 0.76). The poorer relationship observed in the whole shoot data resulted in overlap between the distributions of the genetic classes. In spite of this, the fresh and dry shoot weights of the BC_3F_2 derived lines were more closely correlated with the Na concentration in the whole shoot than they were with the concentration in the YEB. This suggests that factors apart from the Na exclusion gene of Na49 affected Na accumulation in the whole shoot and that these unknown factor/s also affected shoot weight, either directly, or as a result of the change in Na concentration.

The correlations between Na concentration (whole shoot or YEB) and the concentrations of macronutrients were negative, as those observed in the pot and field experiments described in section 2 of this Chapter. On the other hand, the significant micronutrient correlations were all positive, apparently refuting the hypothesis that Na exclusion would result in improved uptake of the micronutrients.

One explanation for the correlation between the concentration of Na and the micronutrients in the shoot (Table 8.11) and YEB (Table 8.13), was that the lines that had accumulated lower concentrations of Na in the leaves had more rapid growth, which led to a dilution of nutrients in the plant tops. At the termination of the experiment, the plants had been growing in 150mM NaCl for only fifteen days. Presumably, the difference in growth rate between high and low Na plants developed in the latter part of the experimental period and was reflected by reduced expansion of the youngest blade. This resulted in accumulation of higher concentrations of all elements.

To test this hypothesis, the correlations between the whole plant Na content and the content of other elements were calculated. These reflected the correlations calculated on the concentrations, refuting the hypothesis that the lower concentration of Zn and Cu in the Na excluding plants resulted from a dilution effect in faster growing plants and indicating that the non-excluding plants did take up more Zn and Cu per plant.

It is possible that the effect of Na exclusion on micronutrient uptake may vary according to the salt concentrations in the substrate. The increased accumulation of Zn in Na excluding plants was observed in a pot experiment with a mean Na concentration of 5,500 mg/kg, whereas the mean Na concentration in whole plants in this solution culture experiment was 25,100 mg/kg, which would have had a much greater effect on plant physiology. Future, experiments investigating the effect of Na exclusion on trace element uptake should include several salt concentrations between 0 and 150mM NaCl.

8.4 The effect of Na exclusion on the accumulation of Na and other elements in grain

8.4.1 Introduction

Backcrossing is a very effective way to introgress a new trait into a range of parents and the selection method described in Chapter 5 was shown to be an accurate and efficient tool in such a breeding program. However, once the trait is common amongst parental lines backcrossing becomes superfluous. At this point, a large portion of the progeny will possess the trait and if it is an important adaptational trait, much of the selection could occur subliminally through yield selection. When the trait becomes common, characterisation of lines at later stages of the breeding program, after selection for other

traits, becomes more important than screening of unselected segregating material. This data, along with other attributes, must be evaluated when making selection or promotion decisions, or choosing parents for further crossing programs.

A method that identifies Na excluders, without the use of an additional selection procedure, is very desirable in this situation. The question arises, is Na exclusion reflected in the Na concentration of the grain? If this is so, the grain from yield trials will provide a much more cost-effective tissue for Na testing, as other yield, agronomy, disease and quality information can be utilised after harvest to reduce the numbers analysed by ICP spectrometry.

8.4.2 Materials and methods

Seven BC_3F_2 derived lines were selected at random from each of the homogeneous low, heterogeneous and homogeneous high Na classes for analysis of the grain harvested from the Redhill and Two Wells sites. These lines also varied in B tolerance, so boron tolerant, intolerant and segregating classes were also represented (Table 8.14).

At each site, two replicates of each line were sampled, as well as six replicates of Kalka and the low Na BC_1 derived line (Kalka#*Na49)/2/85. The six replicates of the two standard lines were chosen by dividing each experiment into six blocks and randomly selecting a plot of each line within each block. These were used as a parental comparison and to provide a measure of spatial variation at each site. All samples were analysed for elemental composition by ICP spectrometry.

Table 8.14. Classification on the basis of Na exclusion and boron tolerance of 21 BC_3F_2 derived lines selected for ICP spectrometry analysis of grain sampled from Redhill and Two Wells, 2003.

Boron Tolerance	Sodium exclusion			Total
	Na excluders	Segregating	Non-excluders	
Tolerant	6	4	4	14
Segregating	0	2	2	4
Intolerant	1	1	1	3
Total	7	7	7	

The Na and B data obtained from the Kalka and (Kalka#*Na49)/2/85 grain was subjected to analysis of variance using Genstat Edition 6 to test for variation between replicates. Correlation coefficients were calculated for the relationships between Na and other elements in the grain of the BC_3F_2 derived progeny using Excel 2000.

8.4.3 Results

Boron

The concentrations of B in the grain at Redhill were below the level that allowed accurate detection by ICP spectrometry.

Analysis of variance of the B concentration of the grain harvested from the BC_3F_2 derived lines grown at Two Wells indicated that the B tolerance classification (tolerant, segregating, or intolerant) was highly significant ($P<0.001$). This relationship resulted in a significant ($P<0.001$) negative correlation ($r = -0.755$) between root length in boron toxic

solution (see Chapter 7, Figure 7.1) and boron concentration in the grain harvested from Two Wells.

Sodium

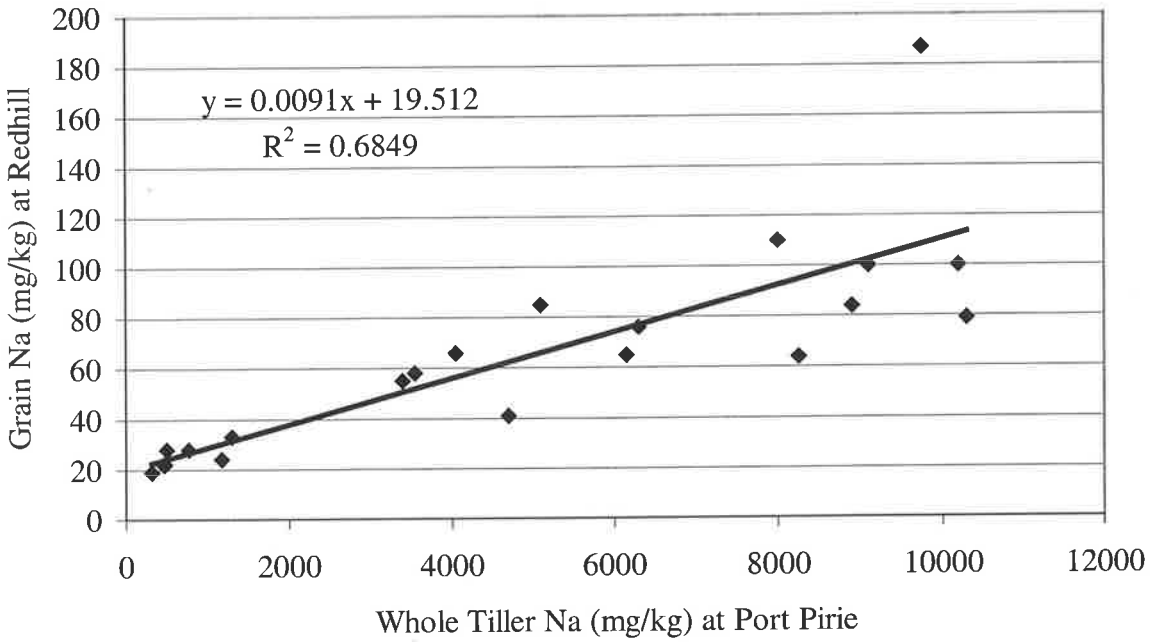
An analysis of variance was undertaken on the Kalka and (Kalka#*Na49)/2/85 data. This revealed that there was no significant effect of replicate on the concentration of Na in the grain at either site, while genotype did have a significant effect at both Two Wells and Redhill ($P<0.01$ and $P<0.01$ respectively). The mean Na concentrations of Kalka and (Kalka#*Na49)/2/85 were 306 ±63 and 125 ±56 mg/kg at Two Wells and 121 ±44 and 24 ±4.6 mg/kg at Redhill respectively.

Analysis of variance of the Na concentration of the grain from the BC_3F_2 derived lines indicated that the Na uptake classification (high, segregating, or low) was highly significant ($P<0.001$) at both Redhill and Two Wells (Figure 8.11). Higher Na classification based on Na in tissue was reflected in greater Na concentration in the grain (Figure 8.13).

Correlation coefficients were calculated between Na concentration in the grain and the concentration of other elements at both sites. Copper was negatively correlated with Na ($P<0.001$) at both sites (Figure 8.14). The only other significant correlation was between grain Na concentration and grain calcium at Two Wells ($P<0.001$).

Figure 8.13. The relationship between Na concentration (mg/kg) in whole tillers sampled at Port Pirie and grain in BC_3F_2 derived lines from the cross (Kalka#*Na49). Grain sampled from (a) Redhill and (b) Two Wells (2003).

(a) Relationship between Na in whole tillers sampled at Port Pirie and grain from Redhill.



(b) Relationship between Na in whole tillers grown at Port Pirie and grain from Two Wells.

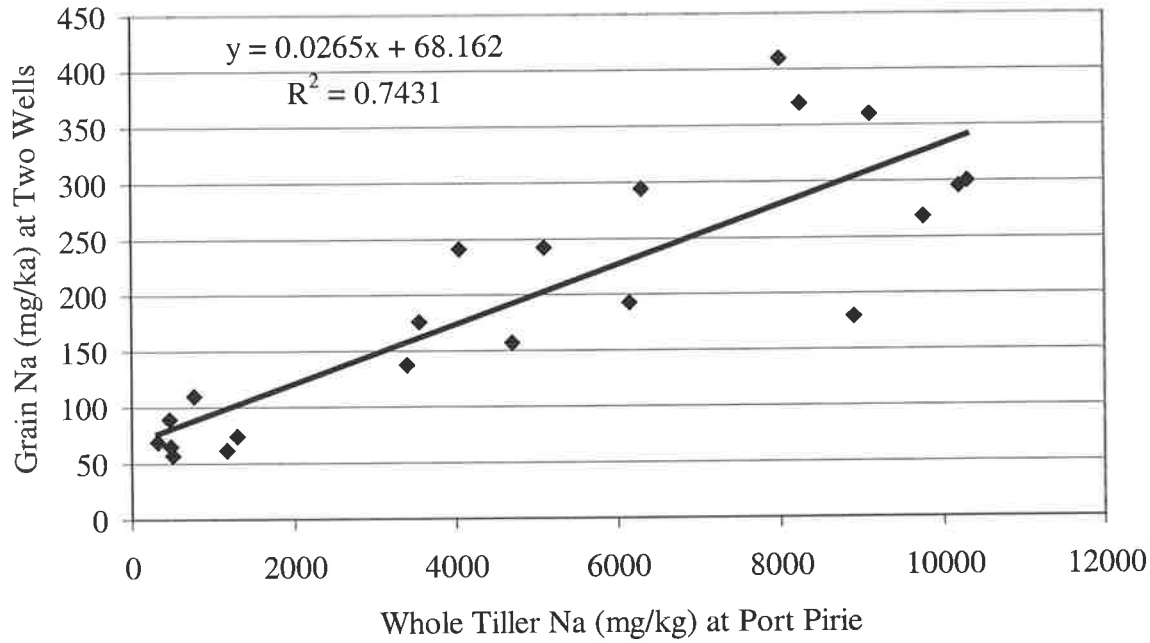
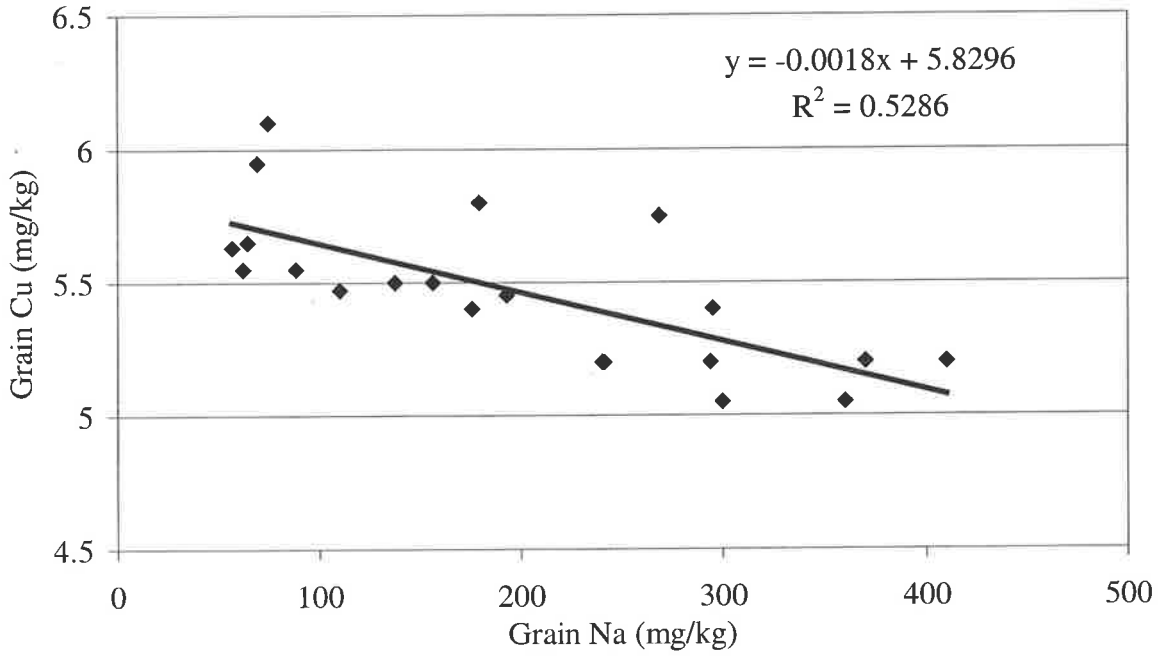
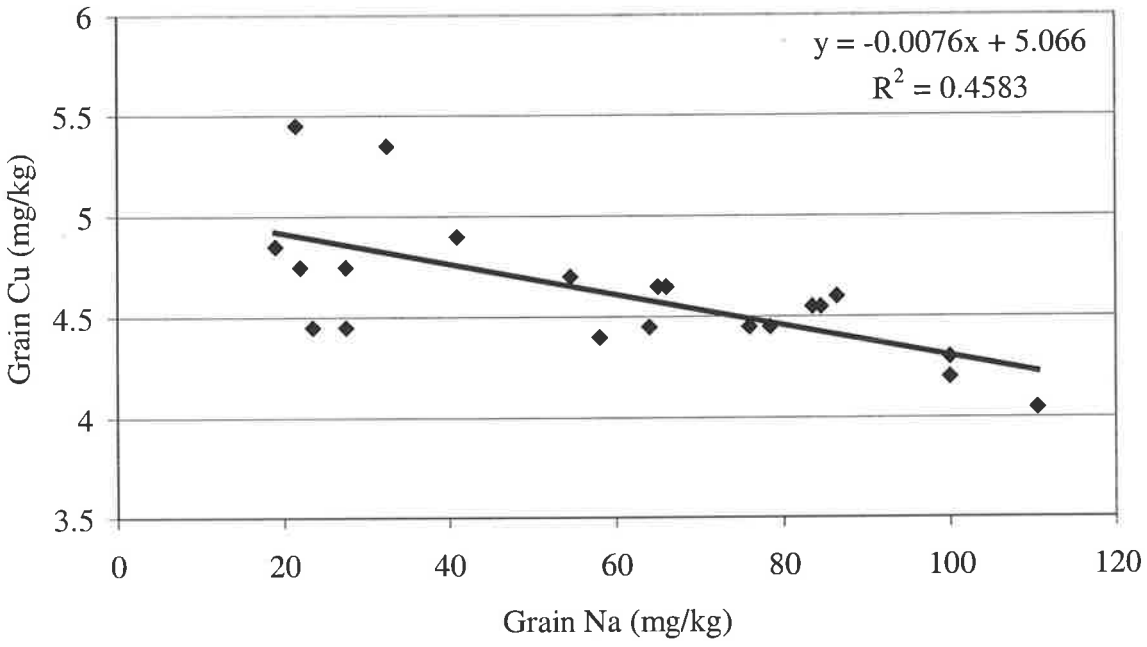


Figure 8.14. The relationship between Na and Cu concentration (mg/kg) in the grain of BC_3F_2 derived lines from the cross (Kalka#4*Na49). Grain samples from (a) Two Wells and (b) Redhill.

(a) Relationship between Na and Cu in Grain sampled at Two Wells



(b) Relationship between Na and Cu in Grain sampled at Redhill



8.4.4 Discussion

These results indicate that the differences in grain Na concentration conferred by the Na exclusion gene, were large enough to allow efficient selection at these two field sites. At the two sites used in this study, the classification of the segregating/heterogeneous and the homogeneous high Na classes were somewhat ambiguous, but no misclassification occurred between the low and segregating classes.

Analysis of grain from more sites will be required to confirm that the high level of accuracy observed in this data is achievable at more variable sites, or sites with low levels of salinity. Nevertheless, the Redhill site does provide evidence that good genetic distinction can be observed at low concentrations of Na. The mean Na concentration in the grain from Redhill was less than half of that measured in the Two Wells grain, with no loss of resolution.

The observation that the concentration of copper in the grain is negatively correlated with Na concentration at Redhill and Two Wells was unexpected, considering that no such correlation had been observed in the whole tiller samples. This suggests that the genotypic differences in grain concentration are likely to be due to an effect on remobilisation. Remobilisation of mineral nutrients is important during reproductive growth, as uptake by the roots generally decreases (Marschner, 1988). Hill *et al.* (1978) found that the leaves of wheat plants with high Cu contents exported more than 70% of their Cu, while the leaves of deficient plants exported 20%. No evidence of an inhibition of Cu remobilisation by high concentrations of Na appears in literature; however this appears to be the most likely explanation for the differences in Cu concentration measured in this experiment.

8.5 Discussion of Chapter 8.

The results of these experiments indicate that, while the accumulation of K in plant tops is significantly higher in Na excluding genotypes, it is not the only element to be affected as suggested in the literature (Dvorak, 1994; Gorham, 1988; Gorham *et al.*, 1987; Greenway and Rogers, 1963; Storey *et al.*, 1985). The large reduction in Na uptake conferred by the locus of Na49 would cause an ionic imbalance within the plant, if not compensated for by the increased uptake of other cations and the reduced uptake of the anions SO_4^{2-} and Cl^-. The largest portion of the change in Na was accounted for by the changes in K and Cl. However, the change in concentration of other elements was not disproportionate when expressed as a percentage of their actual concentration (Table 8.6).

The hypothesis that the Na exclusion of Na49 results from discrimination between the uptake of Na and K (Munns *et al.* 2000) was based primarily on the presumption that the locus was homoeologous to the *Kna1* locus identified by Dvorak *et al.* (1994). While this study did not investigate the effect of Na exclusion conferred by the *Kna1* locus on the uptake of other ions, the discrepancies between the changes in Na and K concentrations published (Gorham *et al.,* 1987; Gorham, 1988; Dvorak *et al.,* 1994), strongly suggest that compensation by ions other than K^+ also occurs in response to Na^+ exclusion by *Kna1*.

Chloride ions were not detectable by ICP-spectrometry. Like Na and K, the concentrations of Cl in the soil solution are also high. The uptake of Cl was also shown to be greatly reduced in response to Na exclusion at Two Wells and it could be expected that this relationship would have occurred in other experiments had Cl been measured.

The increased uptake of other cations in lines carrying the Na exclusion character has the potential to be the difference between deficient and adequate nutrition. The calcium concentration of the Na excluding lines and non-excluding lines was 2154 and 1898 mg/kg respectively at Port Pirie (Data set E). These two concentrations were below the critical value of 2500mg/kg quoted by Reuter *et al.* (1997), but Ca deficiency symptoms were not observed, presumably obscured by the prevalence of crown rot symptoms at that site. Ca deficiency symptoms had previously been observed in Tamaroi field plots on a saline site at Jamestown, while neighbouring bread wheat plots were not visibly affected. ICP analysis of whole tillers of the forty-eight Tamaroi check plots in the Jamestown experiment revealed that the Ca concentration ranged from 1270 to 2900 mg/kg, straddling the critical value of 2500 mg/kg (Reuter *et al.,* 1997). The Jamestown result was analogous to the Port Pirie observation, except that other environmental factors are probably responsible for masking expression of symptoms at Port Pirie and not at Jamestown.

Similarly, the change in K concentration in response to Na exclusion potentially explains some of the lack of adaptation of durum wheat to South Australian soils. At all three field sites sampled, the K concentrations in the high Na accumulating durum lines were in the marginal to deficient range, according to the critical value of 18000 mg/kg quoted by Reuter *et al.* (1997). Historically, K deficiency has not been recognised as a problem in the cereal cropping zone of South Australia, and consequently K fertilisers have not been used. Nonetheless, Mr. S. Roennfeldt identified K deficiency at Laura and Greenock in South Australia in recent years and work conducted at Laura by Wilhelm (2002), has shown that deficiency can occur, which can be ameliorated by the application of K fertiliser. Wilhelm (2002) warned that K reserves were being depleted, particularly where intensive cropping has been undertaken and that deficiency was likely to become increasingly common. The

large reduction in K uptake associated with the high Na accumulation of the current durum varieties may result in K deficiency on soils where bread wheat is not affected. Under these circumstances, durum will have lower than expected yields and probably higher screenings percentages.

The relationship between magnesium and Na uptake was similar to the relationships between Na and both K and Ca. Reuter, *et al.* (1997) found the critical value for Mg concentration to be between 1300 and 1500 mg/kg. While Mg was marginal to deficient in both of the pot experiments (Data sets B and C), it was apparently adequate at the field sites tested. Despite this fact, it is possible that deficiency could occur in durum wheat at other locations, especially as magnesium levels in South Australian soils are commonly low (Holloway and Wilhelm pers comm.). The relationships observed in this genetic population between Na and the uptake of other macronutrients demonstrate that these genotypes have the potential to become a very useful tool for studying the distribution of K, Mg, Ca and S deficiencies.

While the effect of Na exclusion on the uptake of the cationic macronutrients has repeatedly been shown to be positive, the relationship with the uptake of micronutrients is less predictable. An increase in Zn uptake observed in a pot experiment in response to Na exclusion in the glasshouse (Figure 8.5), suggested that the uptake of the micronutrients would also be enhanced by Na exclusion. Supporting evidence was provided by the significantly higher copper concentration in the grain of Na excluding lines at both Redhill and Two Wells (Figure 8.14), although it was likely that this was related to remobilisation rather than root uptake.

The solution culture experiment conducted to investigate further the effect of Na exclusion on the uptake of micronutrients under uniform conditions unexpectedly showed significant positive correlations between the concentration of Na and the micronutrients Cu, Fe, Mn and Zn in plant tissue (Table 8.12), indicating that Na exclusion actually resulted in a reduction in the uptake of micronutrients. The correlations between the whole plant content (as opposed to concentration) of Na and micronutrients confirmed that a decrease in micronutrient uptake had occurred in response to Na exclusion. This refutes the hypothesis that Na exclusion would result in an increase in the uptake of cationic micronutrients.

The concentration of Na in the grain of BC_3F_2 progeny from the cross (Kalka#4*Na49) was shown to be an accurate indication of the Na excluding ability of plants at the two sites analysed. While some ambiguity did occur between the distributions of the homogeneous non-excluding and heterogeneous classes, the homogeneous Na excluding class was distinct. While, this classification may be less accurate at sites with more variable soil salinity levels, the Redhill site provided evidence that good genetic distinction was possible at low Na levels. This result validates grain analysis as an accurate method for identifying Na excluding genotypes, providing flexibility to breeders in their approach to selection and thereby enhancing the breeding of new, salt tolerant varieties.

Chapter 9.

TOLERANCE TO HIGH INTERNAL CONCENTRATIONS OF Na IN A SYNTHETIC HEXAPLOID (*TRITICUM AESTIVUM*)

9.1 Introduction.

There are two main mechanisms of tolerance to high levels of salt. The first is achieved by maintaining a low rate of transport of salt to the shoots by either exclusion or efflux from the root. The second is achieved by allowing salts to move into the plant, reducing the osmotic deficit between the soil and plant, but then partitioning the salt into cell vacuoles (Flowers *et al.,* 1977; Greenway and Munns, 1980). By partitioning the salt into cell vacuoles the impact of salt on cell metabolism is greatly reduced. Typically, salt tolerant wheat genotypes utilise the exclusion mechanism, while barley genotypes take up large quantities of salt, but then partition it to the vacuoles (Greenway and Munns, 1980).

While the research reported in this thesis has focussed on utilising the genetic variation in Na exclusion identified in wheat, future work should investigate whether the vacuole partitioning mechanisms have an additive effect to the tolerance of genotypes carrying the Na exclusion mechanisms of bread wheat or that identified in the durum landrace Na49.

9.2. Tolerance to high internal Na concentrations identified in a synthetic hexaploid wheat.

9.2.1 Introduction

Improved tissue tolerance to high salt levels within the plant has the potential to be a useful addition to salt tolerance conferred by Na exclusion.

Dr. Richard Trethowan, of CIMMYT, observed two synthetic hexaploid wheat (*Triticum aestivum*) lines that appeared to be better adapted to irrigated saline soils. These two lines were derived from a cross between the durum variety Ceta and an accession of *Aegilops squarosa*. Another synthetic hexaploid wheat, which had been observed to be more tolerant of drought than other bread wheat lines, was also included in this study. The two purported salt tolerant synthetic wheat lines are referred to by their Australian Winter Cereals Collection accession numbers Aus#29663 and Aus#29664, while the drought tolerant line is designated Aus#29503.

9.2.2 Materials and methods

The experiment consisted of five genotypes (Aus#29503, Aus#29663, Aus#29664, Krichauff and Kharchia 65), and five salt treatments (including a control), which were combined factorially and replicated three times in randomised blocks on a glasshouse bench. Each entry was represented by a single pot containing two plants.

Seed

The seed was surface sterilised in 2% sodium hypochlorite, rinsed and pre-germinated on filter paper in Petri dishes for four days at 4°C, followed by two days at 20°C.

Growth media

The growth media was modified from the method of Maher *et al.* (2003). Plants were grown in 170mm diameter pots containing 2.1kg of free draining dried coarse river sand from Waikerie, which has a water holding capacity of 19%. The pots were watered every second day with 500mL of dilute commercial nutrient solution, (Nitrosol ®), at twenty

percent of the recommended concentration, and 10mM $Ca(NO_3)_2$, in mains water. The irrigation volume of 500mL was chosen deliberately to be in excess of the water holding capacity (399mL), so that the excess solution flushed the pot, leaching out any salt which had accumulated through evaporation or water uptake by the plants.

Salt treatments

Each genotype was subjected to five final salt treatments (Control, 37.5, 75, 112.5 and 150mmol/L). The salt treatments were added to the base solution from ten days after plant emergence and the higher salt treatments were increased incrementally by 37.5mmol NaCl every four days, until the planned concentrations had been reached.

Data collection

Heading time was similar for all varieties, so all genotypes were scored and harvested at the same time, without concern for maturity effects. The percentage of green area of the flag leaf, first leaf below the flag and the second leaf below the flag was estimated using a ruler marked with centimetre graduations. Most of the lower leaves (below the third from the flag) had senesced. The green area of each leaf was averaged for the two plants within each pot. Green leaf estimations were made without knowledge of genotype, or salt treatment, to reduce the chance of any bias. The number of tillers were counted and mean calculated.

The whole shoots were cut at ground level and the fresh weights recorded (bulk of two plants from each pot), before being washed in RO water, dried at 80°C for 24 hours and weighed. The dry shoots were chopped finely using stainless steel scissors and submitted

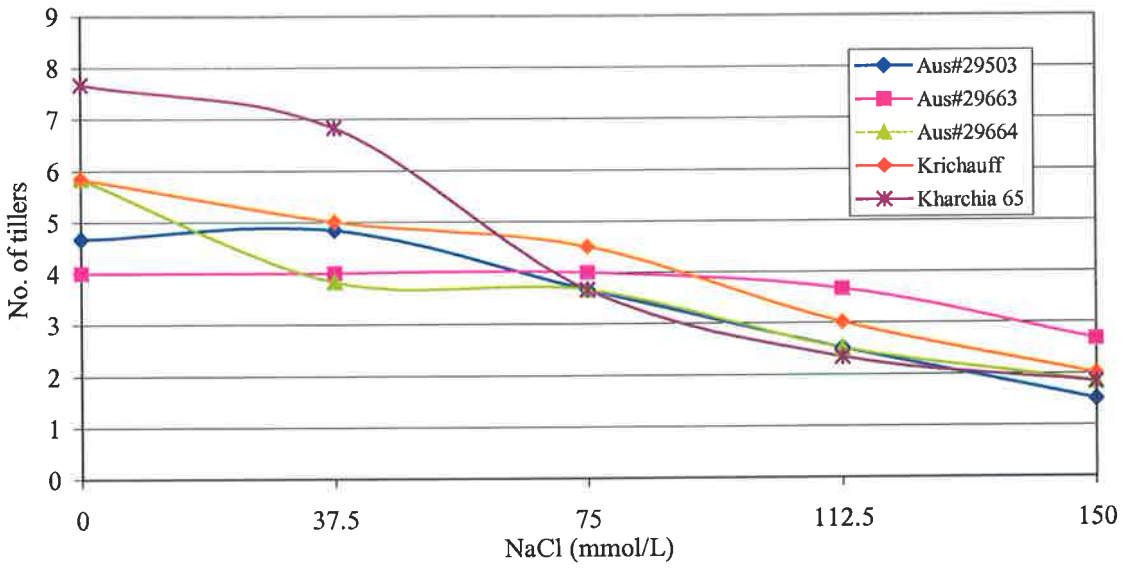
to Waite Analytical Services for analysis of elemental composition using ICP-spectrometry.

9.2.3 Results

Number of tillers

The effect of genotype (P<0.001), salt treatment (P<0.001) and their interaction (P<0.001) were all significant on the number of tillers per plant (Figure 9.1). The genotypes varied for the number of tillers in the absence of salt (4 to 7.7 tillers/plant) and the effect of increasing salt concentration was to reduce this range. It is also clear that Aus#29663 had the smallest number of tillers per plant in the absence of salt, but that there was no reduction in tiller number up to a NaCl concentration of 112.5mmol/L, at which concentration it had the largest number of tillers.

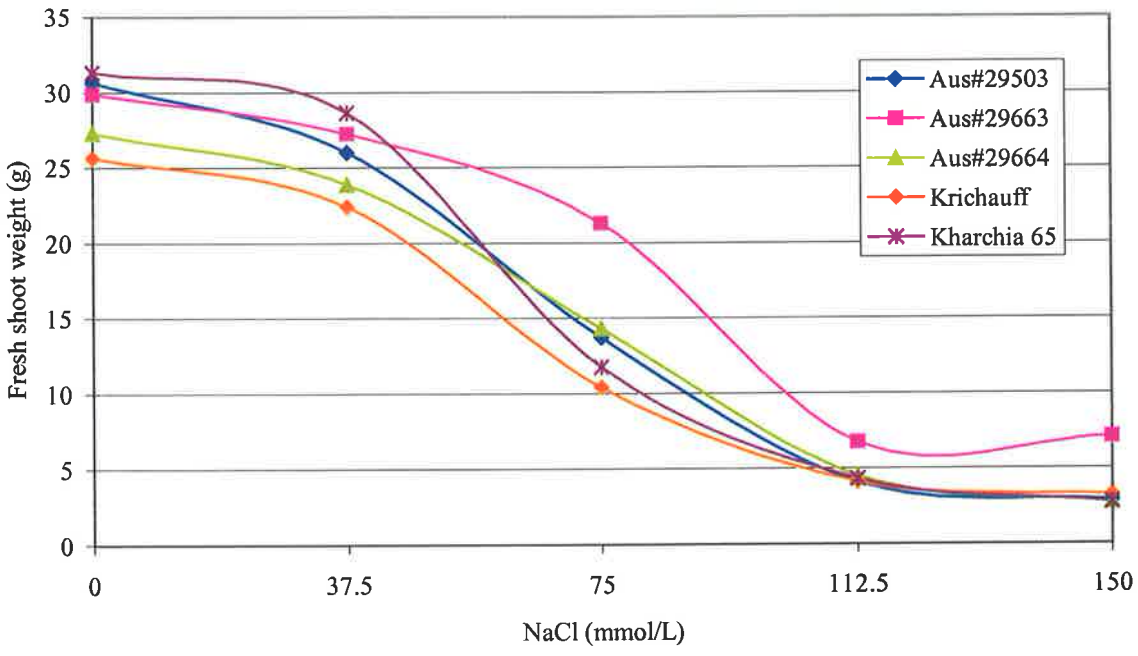
Figure 9.1. The effect of NaCl concentration on the number of tillers per plant of four genotypes. Plants were irrigated with a nutrient solutions containing five NaCl concentrations. LSD (5% level) = 1.06.



Fresh shoot weight

Both genotype and salt treatment had highly significant effects on fresh shoot weight ($P<0.001$), while the interaction between them was significant at the 5% level of confidence. The effect of increasing salt concentration had a similar effect on the fresh shoot weight of all genotypes, except for Aus#29663, which maintained a higher shoot weight with the addition of salt, particularly at the 75mmol/L treatment (Figure 9.2). This interaction resulted in the fresh shoot weight of Aus#29663 being reduced by only 22% between the 37.5 and 75mmol/L treatments, compared with 40 to 59% reductions in the other genotypes.

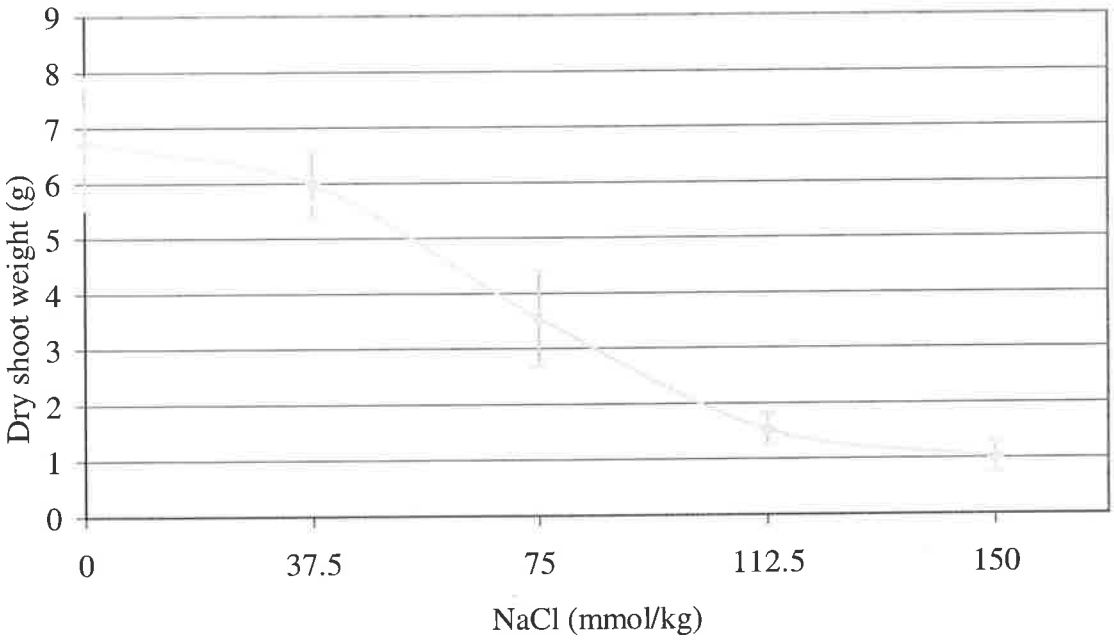
Figure 9.2. The effect of genotype and NaCl concentration on the fresh shoot weight of wheat plants. LSD (5% level) = 4.087.



Dry shoot weight

Salt concentration was the only variable to significantly effect dry shoot weight ($P<0.001$). The effect of increasing salt concentration on plant weight was nearly linear between 37.5 and 112.5mmol/L, while above this range, most plants were either approaching, or actually dead, so that no further reduction in plant growth was possible (Figure 9.3).

Figure 9.3. The effect of NaCl concentration on the mean dry shoot weight of five genotypes of bread wheat at Zadocks growth stage five (head emergence).



Percent of green leaf area

Salt concentration, genotype and the interaction between them all had significant ($P<0.001$) effect on the green leaf area of the flag leaf, as well as the first and second leaves below the flag. This interaction was most clearly observed in the 37.5 and 75mmol/L NaCl treatments, where the green area of the flag leaf of Aus#29663 was reduced by only 8.3% compared to 34 to 76% reductions for the other genotypes (Figure 9.4).

The green area of the first and second leaves below the flag leaves of Aus#29663 was much greater than that of other genotypes at the 37.5 and 75mmol/L salt concentrations, but unlike the flag leaf estimates, the percentage reduction in green area was not a suitable measure of tolerance. Intolerant genotypes, which had less than 50% green leaf area at the 37.5mmol/L concentration, tended to suffer smaller reductions (%) between the 37.5 and 75mmol/L concentrations (Figure 9.4).

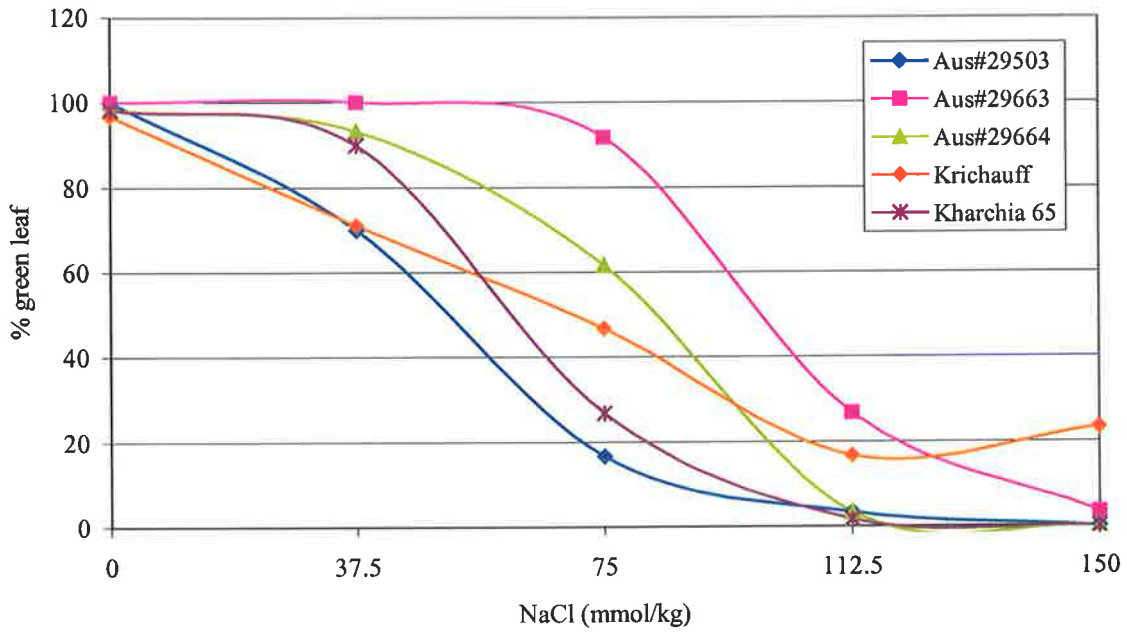
Figure 9.4. The effect of genotype and NaCl concentration on the percentage of green leaf area at head emergence (Zadocks growth stage 5) of five genotypes irrigated with saline nutrient solution in pots in a glasshouse.

(a) Flag leaf;

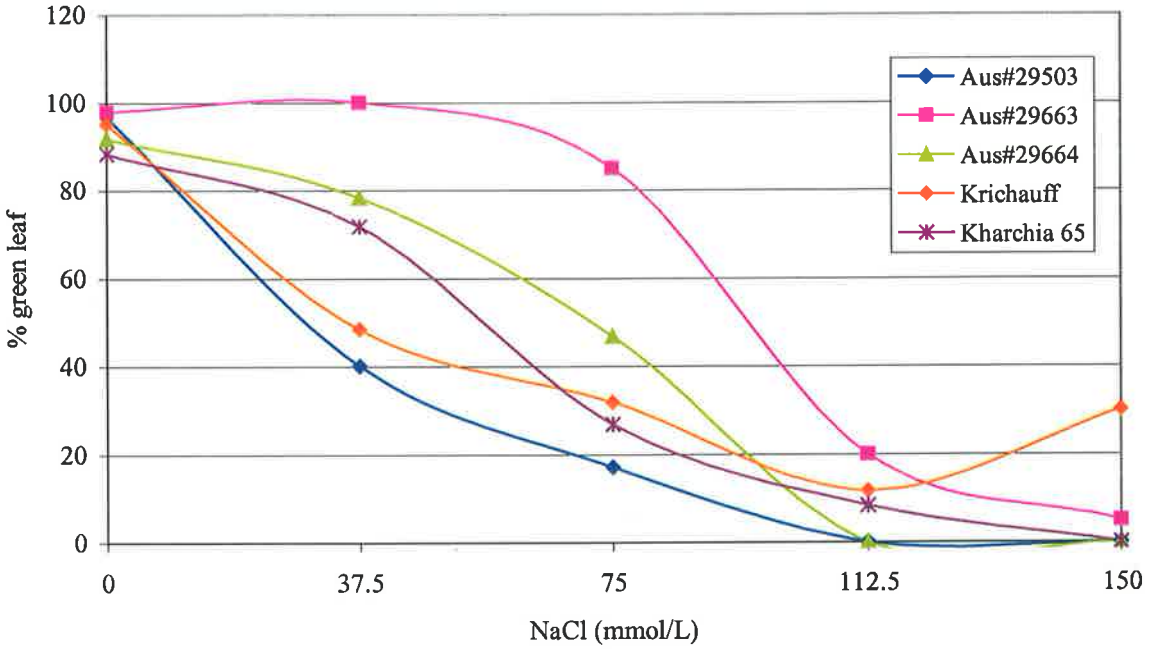
(b) First leaf below the flag and;

(c) Second leaf below the flag.

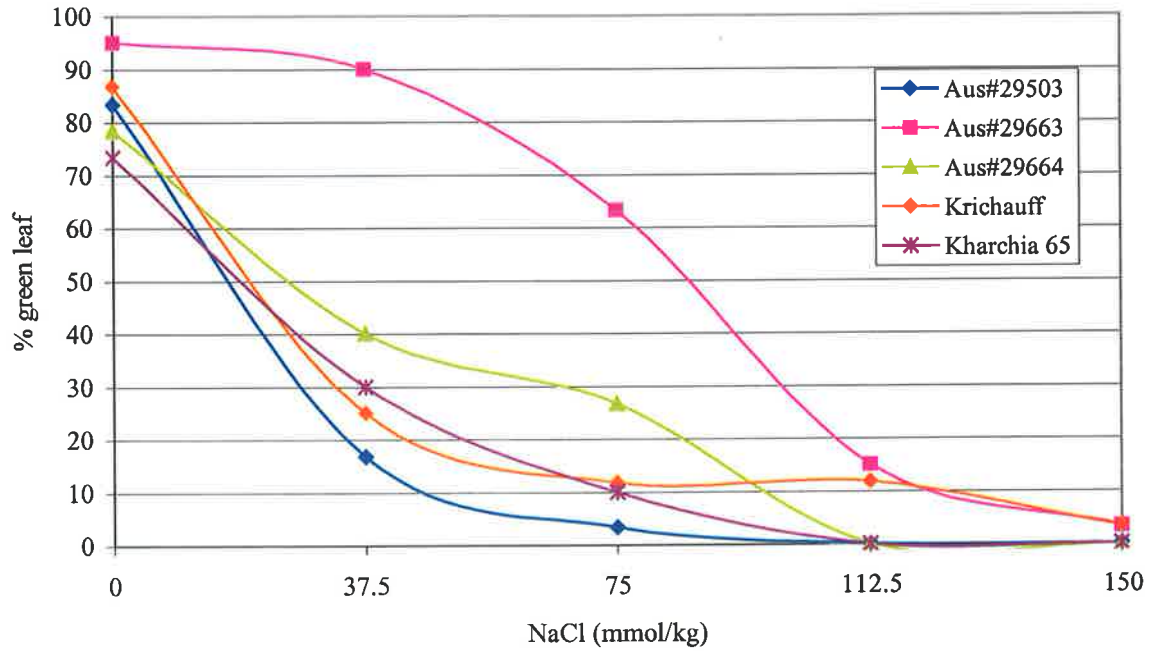
(a) Percentage of green flag leaf. LSD (5% level) = 20.3.



(b) Percentage of green area of the first leaf below the flag. LSD (5% level) = 17.8.



(c) Percentage of green area of the second leaf below the flag. LSD (5% level) = 19.0.



The differences in green leaf area and fresh shoot weight of plants grown in the 75mmol/L treatment is evident in the photograph (Plate 9.1). Similarly, the reduction in green leaf

area of Krichauff over the range of 37.5 to 112.5mmol/L was clearly far greater than that of Aus#29663 (Plate 9.2).

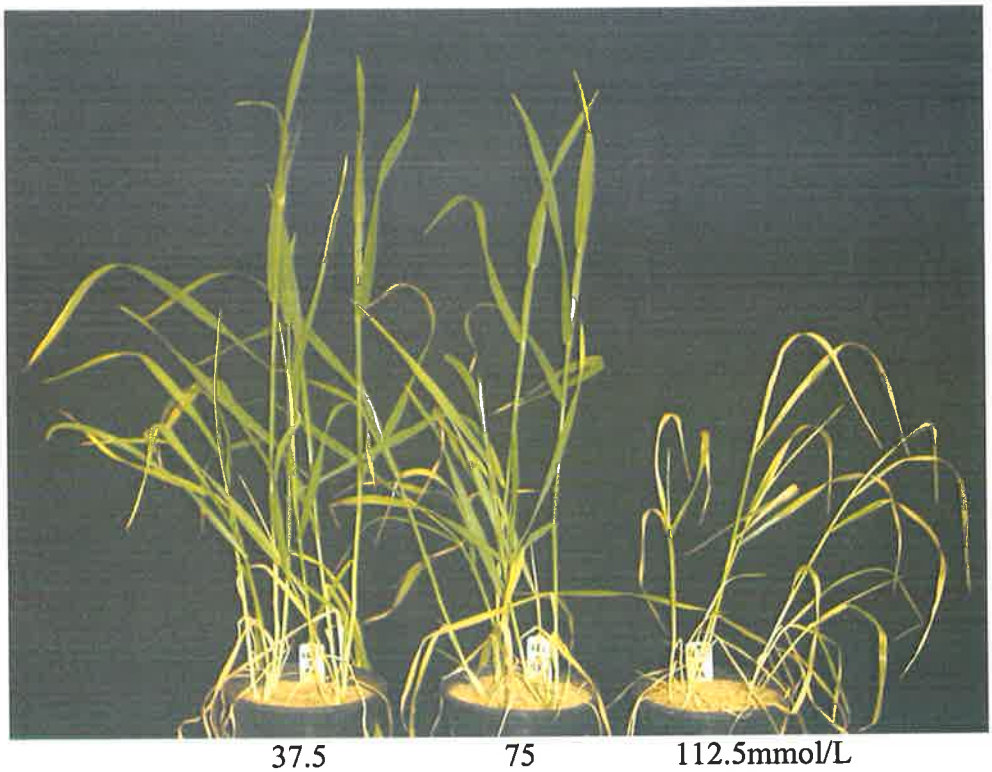
Plate 9.1. The genotypes Kharchia 65, Krichauff and Aus#29663, irrigated with nutrient solution containing 75mmol/L of NaCl.

Kharchia 65 Krichauff Aus#29663

Plate 9.2. (a) Krichauff wheat irrigated with nutrient solution containing 37.5, 75 and 112.5mmol/L of NaCl.

37.5 75 112.5mmol/L

(b) The synthetic hexaploid wheat Aus#29663 irrigated with nutrient solution containing 37.5, 75 and 112.5mmol/L of NaCl.



37.5 75 112.5mmol/L

Na concentration in the whole shoot

Salt treatment (P<0.001) and the interaction between genotype and salt treatment (P<0.01) had a significant effects on the concentration of Na in the whole shoot, while the effect of genotype was not significant. The interaction was the result of differences in Na concentration up to the 75mmol/L treatment, but at higher concentrations, there was no clear effect of increasing salt concentration on individual genotypes (Figure 9.5). The synthetic hexaploid Aus#29663 had the lowest Na concentration in the 75mmol/L treatment, followed by its sister line Aus# 29664. While it appears that these two lines were able to exclude Na from the shoot, they actually had higher total Na uptake on a whole plant basis (Figure 9.6), indicating that the lower concentrations in the shoot resulted from a dilution effect.

Figure 9.5. The effect of increasing substrate NaCl concentration on Na concentration in the whole shoots of five hexaploid wheat genotypes. LSD (5% level) = 6269.4.

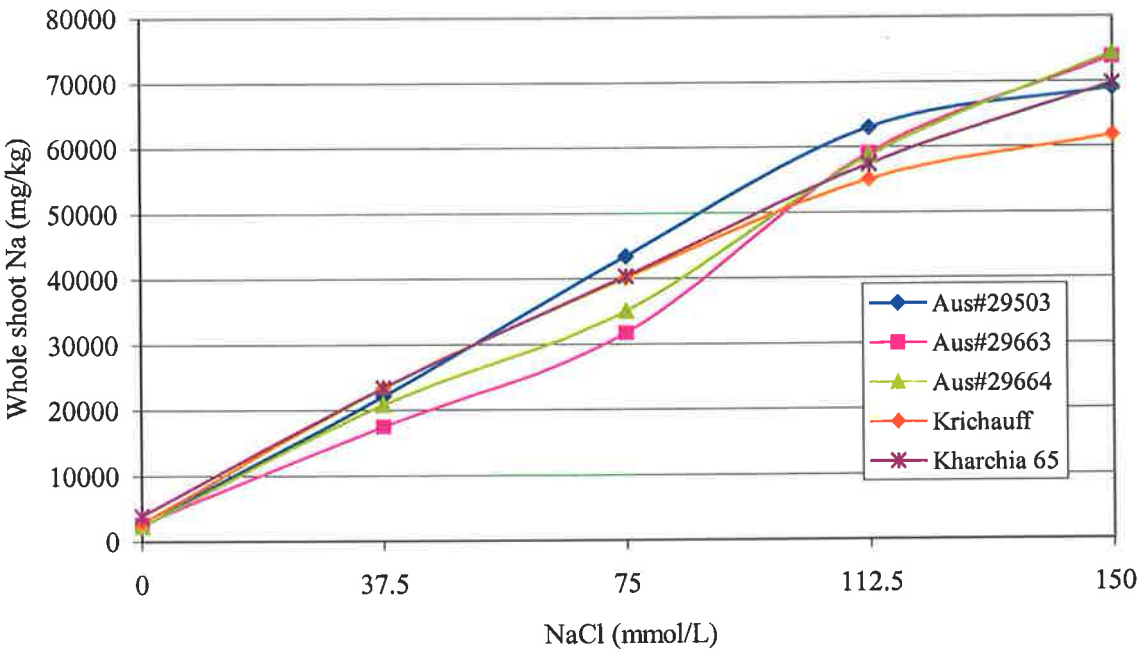
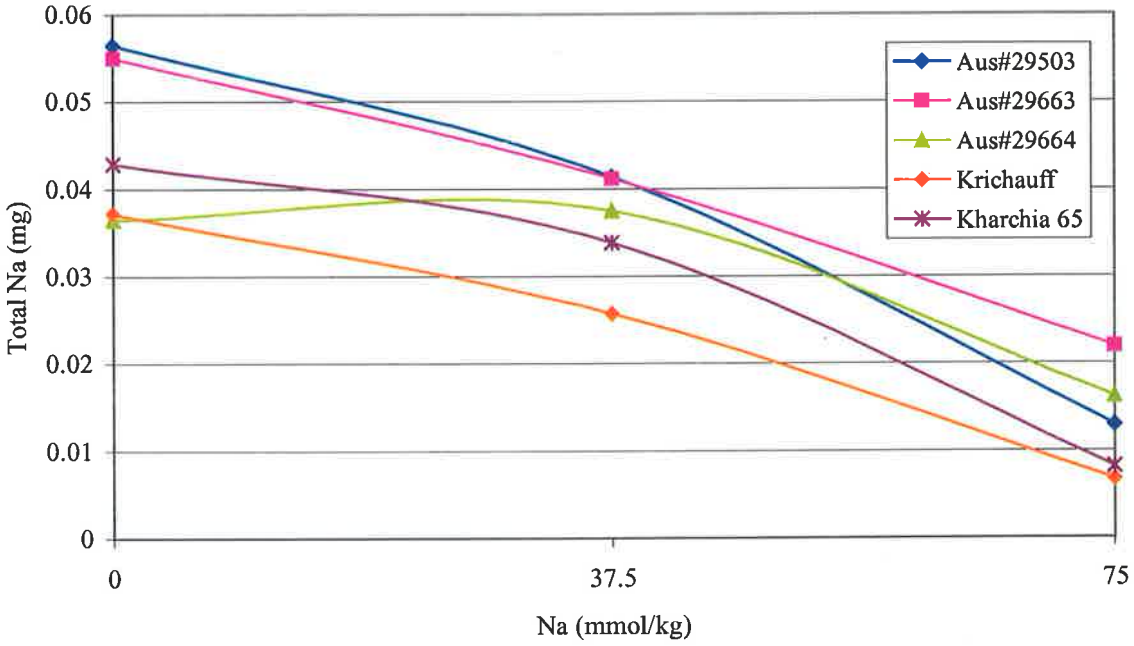


Figure 9.6. The effect of increasing substrate NaCl concentration on the total plant Na uptake of five bread wheat genotypes. Values are for the bulk of two plants.



9.2.4 Discussion

The synthetic hexaploid wheat Aus#29663 was able to retain the photosynthetic area of the top three leaves at much higher NaCl concentrations than other genotypes. This tolerance did not result from an ability to exclude more Na than the other genotypes (Figure 9.6), suggesting that it is able to tolerate higher internal concentrations of Na. The lower internal Na concentration of Aus#29663 up to the 75mmol/L NaCl treatment (Figure 9.5) resulted from this genotype maintaining a higher growth rate, which resulted in a dilution effect of the Na within the plant.

Although the genotypic differences in fresh shoot weight (Figure 9.2) were smaller in magnitude than the differences in green leaf area (Figure 9.4), the genotypic differences in leaf symptoms only developed in the five days leading up to the plants being harvested. As much of the dry matter production would have occurred prior to the development of the

genotypic differences in photosynthetic area, the actual differences in plant growth in the last five days may have been much greater than the shoot weight measured over the duration of the experiment would suggest.

9.3 Salt tolerance of doubled haploid progeny from the cross (Aus#29663*Krichauff)

9.3.1 Introduction

The previous experiment (Section 9.2) identified the synthetic hexaploid wheat Aus#29663 as having a superior level of tolerance to high salt concentrations in the leaves than the locally adapted, Na excluding (see chapter 4) variety Krichauff. While a wide range of genotypes had not been assessed for salt tolerance by this method, Krichauff is highly adapted to transient saline soils in South Australia and has been shown to have the ability to exclude more Na than other varieties (Chapter 4).

The identification of tolerance to high internal Na concentrations in Aus#29663, suggests the possibility of combining this trait with the Na excluding ability of Krichauff to improve further the tolerance of locally adapted genotypes. The most suitable method of achieving this would be to backcross the trait into Krichauff; however, this requires knowledge of the genetic control of the trait.

A population of sixty-two doubled haploid progeny from the cross (Aus29663*Krichauff) developed by Dr. Hugh Wallwork of the CRC for Molecular Plant Breeding and Dr. Neil Howes of SARDI, provided the opportunity to study the genetics of the tissue tolerance trait. It was also hoped that a doubled haploid line carrying the green leaf area trait could be identified to be used as the donor parent in the backcrossing program.

The population was screened for green leaf area using the method described in the previous experiment (Section 9.2). Unfortunately, the amount of seed produced by the doubled haploid plants varied considerably, from two to more than ten seeds. Due to this lack of seed, the plants were screened only using the 75mmol/L treatment, as this provided the best discrimination between the genotypes included in the experiment described in Section 9.2.

9.3.2 Materials and methods

Each replicate consisted of a single pot of two plants. Two replicates were included for each of the doubled haploid lines with adequate seed and these were arranged in two separate blocks. Five replicates of each parental line were also included, three in block one, and two in block two. Where three seeds or less of a doubled haploid line were available, the line was only included in one replicate.

The pots, irrigation solutions, and methods used were the same as those used in the previous experiment for the 75mmol/L treatment.

The very small quantities of seed available meant that it was important to produce as much seed as possible from the plants for further research and breeding. For this reason, fresh and dry weights were not measured and after the observations reported here had been completed, the plants were watered with non-saline nutrient solution until grain maturation.

The experiment was planted in the glasshouse on the ninth of January and discontinued on the sixteenth of February, after two very hot days on the fourteenth (44.3^{o}C) and fifteenth

(42.3°C) killed many plants (maximum temperatures recorded by the Bureau of Meteorology, Kent Town (Adelaide)).

9.3.3 Results

The doubled haploid progeny exhibited far greater variation for many characters than was observed between the two parental lines. Apart from the synthetic parent, Aus#29663, having slightly later maturity, its growth habit was similar to that of Krichauff. In contrast, the progeny exhibited a wide range of maturity, from lines earlier than Krichauff, to lines with very late maturity. When the final observations were made some lines were approaching grain maturation, while others were still in the vegetative stage (Plate 9.3). The doubled haploid lines also varied widely for vigour, height and standing ability.

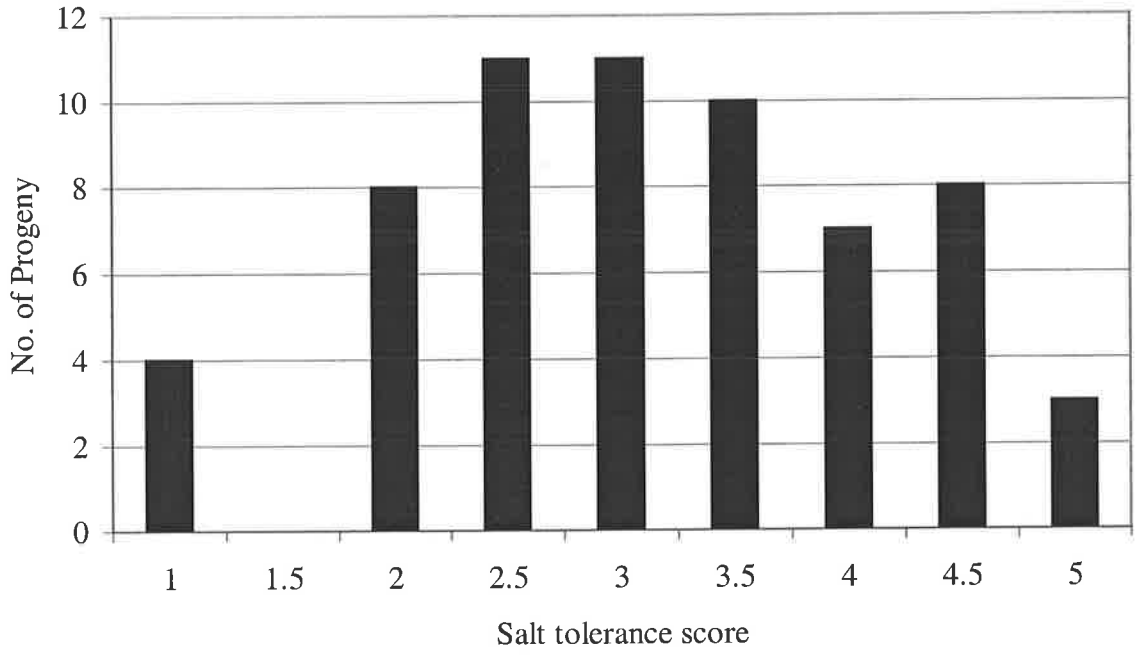
The late maturing lines appeared to suffer from salt toxicity far less than the early lines, which may have been due to either a physiological difference in tolerance dependent on development particularly with respect to the abnormally hot days, or some genetic linkage between maturity and the salt tolerance trait. These factors made direct comparisons on the basis of green leaf area of the top three leaves impossible and reduced the scoring system to a one to five scale based on the general amount of leaf damage suffered by the plant. Some indication of the variation in leaf damage between lines of similar maturity is provided in Plate 9.4.

Plate 9.3. Variation in maturity among a selected group of three doubled haploid progeny derived from a cross between Aus#29663 and Krichauff. Two replicates of each line were included in the photograph.

Plate 9.4. Variation in salt tolerance observed between doubled haploid progeny derived from the cross between Aus#29663 and Krichauff. Lines selected on the basis of similarity of maturity.

The difference in salt tolerance between the parental lines Aus#29663 and Krichauff was not as great as was observed in the previous experiment. On the scale of one (tolerant) to five (intolerant), Aus#29663 had a score of 2.5, while Krichauff had a mean score of 3.8. The tolerance scores of the doubled haploid progeny ranged from one to five in a continuous distribution (Figure 9.7). As clearly defined classes could not be identified, no attempt was made to define the mode of inheritance of the trait, particularly as the variation in maturity appeared to be correlated with the salt tolerance scores.

Figure 9.7. Distribution of sixty-two doubled haploid progeny from the cross between Aus#29663 and Krichauff, for a visual score of tolerance to irrigation solution containing 75mmol/L of NaCl. Scores were based on the level of leaf damage, where a score of one was tolerant and five intolerant.



9.3.4 Discussion

Variation for salt tolerance was observed within the population derived from the cross between Aus#29663 and Krichauff; however, the wide range of maturity, combined with

the low number of replicates (either one or two), make accurate classification of the lines on the basis of tolerance inaccurate. Consequently, more extensive experiments need to be undertaken before any conclusions can be drawn regarding the genetic control of the trait.

The wide range in maturity appeared to be at least partly due to variation in day length sensitivity, as the very early lines initiated reproductive growth in the long days of January without any discernable vegetative phase. If day length sensitivity was responsible for the variation in maturity, it may have been possible to reduce the range of variation by conducting the experiment in winter and early spring, when the day length is short and lengthening. Under these conditions, the initiation of reproductive growth in early maturing lines with day length sensitivity would be delayed.

9.4 Discussion of Chapter 9

The synthetic hexaploid wheat, Aus#29663, was shown to tolerate higher substrate concentrations of NaCl than Kharchia 65, or the locally adapted genotype, Krichauff. While the Na concentration of Aus#29663 was lower than that of Krichauff when grown in NaCl solution up to a concentration of 75mmol/L, its total plant uptake was higher, suggesting that its higher level of tolerance was achieved without an additional level of Na exclusion over that of Krichauff. The lower internal Na concentration of Aus#29663 presumably resulted from a dilution effect within the faster growing, more tolerant plant, which was able to continue to photosynthesise despite very high internal Na concentrations.

A possible explanation for this tolerance is that this genotype has the ability to sequester Na to cell vacuoles, alleviating toxicity in the cytoplasm. This mechanism is utilised by

many halophytes such as *Suaeda maritima,* which has been shown in one study to have a concentration of 150mM Na^+ in the cytoplasm and 600mM in the vacuole (Yeo, 1974). This mechanism is also suggested to be responsible for the tolerance of barley, which accumulates much higher concentrations of Na than wheat when grown under the same conditions, but exhibits tolerance at least equivalent to wheat (Munns, pers. comm.). The only way to confirm that this mechanism was responsible for the tolerance of Aus#29663 is to measure the vacuole and cytoplasmic Na concentrations of Aus#29663 and other less tolerant genotypes.

Variation in salt tolerance was observed among the doubled haploid progeny derived from the cross between Aus#29663 and Krichauff, but accurate assessments were not possible due to the extreme variation in maturity. While it may be possible to reduce the range of the maturity differences by repeating the experiment in winter and early spring, it is highly likely that the considerable genetic differences between the two parents will make accurate assessment of tolerance difficult.

The lines exhibiting a high degree of salt tolerance in this experiment are currently being backcrossed to Krichauff, in an attempt to reduce the proportion of genetic material from Aus#29663. A population derived from this cross, or subsequent crosses to Krichauff will provide material more suitable for studying the genetic control of the trait, through a reduction in variation for other traits such as maturity.

Chapter 10.

GENERAL DISCUSSION

Crop Adaptation

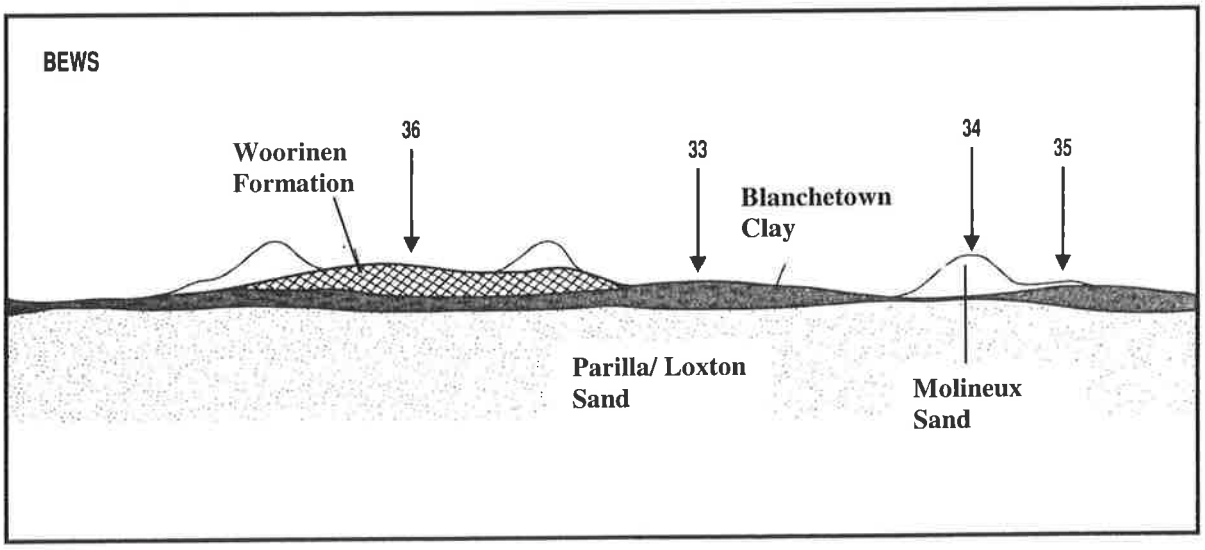
Field experiments were conducted in 2001 and 2002 to investigate the effect of transient salinity on crop growth (Chapter 3). The sites were selected for either high or low levels of transient salinity, without any specific regard for the presence of other subsoil constraints. After harvest intensive soil sampling to a depth of 60cm revealed that some sites, also had toxic concentrations of boron and high pH. Toxic concentrations of boron were present (>3mg/kg) at a depth of 30-40cm and all sites, except Jamestown (2002), had a mean pH in excess of 8.5 below a depth of 30cm. Above this pH, the concentration of free bicarbonate ions increases substantially, while many micronutrients become less available (Peverill *et al.*, 1999; Rengasamy, pers. comm.). This prevalence of high pH subsoils should not have been surprising, as mapping work undertaken by D. Maschmedt and his team has shown that high pH subsoils dominate the agricultural soils of South Australia (PIRSA land information, 2001).

Despite the fact that more than half of the sites were located on land with subsoil (30-40cm) salinity levels in excess of 4dS/m ECe, the transient saline site at Roseworthy was the only location where $EC_{1:5}$ was the statistically dominant covariate affecting yield (Figure 3.7). Soil boron concentration had the dominant effect on yield at the non-saline Roseworthy site, while at three of the six sites investigated in 2001, pH had the most significant impact. This highlights the importance of the subsoil constraints other than transient salinity on field crop production in South Australia.

These results indicate that combining tolerance to the three constraints in new durum and bread wheat varieties will maximise the benefits of any individual tolerance. As an example, at the transient saline Angas Valley site (Figure 3.3), the topsoil (0-10cm) was the best indicator of crop yield, despite the mean salinity and boron concentrations being above their critical values at 30-40cm (Table 3.1). It is highly unlikely that either boron or salt tolerant durum genotypes will have any substantial benefit at this site without also possessing tolerance to high pH.

The Bews System provides a summary of the geology of much of the Murray Mallee, while the cross-sectional diagram (Figure 10.1) explains the coincident occurrence of boron, salt and high pH in the same parts of the landscape.

Figure 10.1. Geological cross-sectional diagram (not to scale) of the Bews system of the South Australian Murray Mallee. Reproduced from McCord (1995).



The Blanchetown Clay, of alluvial origin, is the basis of much of the heavier soils of the Murray Mallee and underlies the Woorinen and Molineux formations which comprise most of the lighter soils. The Woorinen formation originated as a calcareous loess of marine

origin, containing high concentrations of boron, salt and $CaCO_3$. The Blanchetown clay has become more sodic and impervious over time, so that it now forms a barrier to further leaching. Consequently the subsoil constraints studied in this thesis (boron, transient salinity and high pH), plus the K, Mg and Ca deficiencies tend to occur most severely around the junction of the Woorinen and the Blanchetown clay. Meanwhile the areas with deeper Woorinen formation have had the boron and salts largely leached from the surface, leaving high pH and micronutrient deficiencies as the predominant problems. The more recent Molineux sands, reworked soils of the most recent glacial period, are characterised by very low nutrient levels and poor water relations, often becoming non-wetting. The predominance of these formations across the Murray basin, and the equivalent (Wiabuna formation) which covers much of the Eyre Peninsula, explain the poor performance in these areas of current durum varieties, which are intolerant of the subsoil constraints associated with these soils.

The distribution of other broad-acre crops, such as canola, lupins, peas, faba beans, lentils, and to a lesser extent, barley, has also been largely restricted to the soils not affected by this complex of problems. In the areas where these crops have been grown successfully they have been important break crops, reducing the impact of soil borne diseases and pests. So improving the adaptation of these crops to soils affected by the subsoil constraints would improve the flexibility available to growers in the drier areas of southern Australia and improve farm profitability.

Genetic Investigations

The Na excluding durum landrace Na49 (Munns *et al.*, 2000) was used as a donor parent in a backcrossing program to introgress the Na exclusion trait into the background of the boron tolerant variety, Kalka (Chapter 5). A population of 196 F_2 derived lines were selected from the third backcross to Kalka, which were characterised for Na exclusion, multiplied and grown at three field sites in 2003.

At all stages of the backcrossing process, the trait appeared to be conferred by a single dominant gene linked to a chromosome 2A marker identified by Dr. Rana Munns and collaborators. This disagreed with the conclusions of Munns *et al.* (2003) who had concurrently studied a population of 100 F_2 derived lines from the cross (Na49*Tamaroi) and concluded that the trait was controlled by two genes.

On the evidence described in Chapter 6 of this thesis, it appears highly likely that the preferential sheath retention trait and the marker on chromosome 2A co-segregated with the whole plant exclusion trait. Recent work by Tester (unpublished) has shown that there is no difference in influx into the root between Na excluding and non-excluding lines. Therefore, it is highly likely that efflux is the basic mechanism leading to the low Na concentrations in the shoot, and that this results from a restriction on xylem loading, or the promotion of xylem unloading of Na (Tester, pers comm.). Hence, the same gene product could be responsible for promoting unloading of Na from the xylem in both the root and the shoot.

The full recovery of the Na^+ excluding ability of Na49 in the background of Kalka, is adequate for the current breeding requirements. This locus has reduced the Na

concentration in the shoot of durum wheat at least five fold, to a level which is comparable with the concentrations regularly observed in bread wheat. The progeny of this backcrossing program are now being widely tested for commercial release and are being used in new crossing programs.

Combining Na^+ tolerances in search of transgressive segregants

The Na excluding ability of bread wheat is much greater than that of commercial durum varieties. This large difference in the ability to exclude Na is believed to be conferred by the *Kna1* locus on the long arm of chromosome 4D of bread wheat (Dvorak *et al.*, 1994; Dubcovscy *et al.*, 1996), but additional variation has been shown to exist within *T.aestivum* (Chapter 4). Thus, the widely adapted variety Krichauff accumulated half as much Na as compared to its sister variety, Worrakatta, when the two genotypes were grown in saline pots (Table 4.2). While this effect appears to be much smaller than that of either the *Kna1* locus, or that of the locus from Na49, the gene from Krichauff may have an additive effect to both of these other loci.

Bulked segregant analysis indicated that the additional Na excluding ability of Krichauff (over Worrakatta) was controlled by a QTL on the long arm of chromosome 4B, linked to the SSR marker *gwm149*. This locus could be incorporated into the background of durum wheat through crossing and recombination, providing an alternative, or additional source of Na exclusion, to that identified in Na49.

Most of the research described in this thesis has been focused on the effects of Na exclusion on tolerance, but a synthetic hexaploid wheat with tolerance to high internal concentrations of Na was also identified (Chapter 9). The synthetic hexaploid Aus#29663

was able to maintain a higher percentage of green leaf area when grown in pots irrigated with saline nutrient solution than either Kharchia 65, or the locally adapted variety, Krichauff. Although Aus#29663 had a significantly higher fresh shoot weight than other genotypes when grown in 75mmol/L NaCl, ICP analysis showed that, overall, the Na concentration within Aus#29663 was not significantly different to the other genotypes, indicating that it is able to tolerate higher Na concentrations in the shoot.

The existence of the three known sources of Na^+ exclusion, *Kna1*, Na49, and the locus linked to the marker *gwm149* on chromosome 4B of Krichauff, has provided the opportunity to combine multiple sources of exclusion in either durum or bread wheat with the aim of selecting transgressive segregants with low internal Na concentrations, even when grown on highly saline sites. Work is already underway in the CRC for Molecular Plant Breeding to introgress the salt tolerance of Aus#29663 into the genetic background of Krichauff, potentially combining Na exclusion with tolerance to high internal levels in a locally adapted background.

Grain Yields

The toxic effects of elevated levels of Na and Cl ions on metabolism have been investigated by many researchers (Flowers, *et al.*, 1977; Greenway *et al.*, 1981; Jennings, 1976). From this evidence Greenway and Munns (1980) drew the conclusion that reduced uptake, or active exclusion of these ions by plants should improve tolerance. However, direct evidence that Na exclusion confers field tolerance to salinity has not been reported in the literature.

The F_5 derived population of the cross (Worakatta*Krichauff) was sown at four sites in 2003 to assess the benefit conferred by the Na exclusion locus of Krichauff. The Na concentration in whole tillers (sampled at Redhill) was correlated with grain yield only at Buckleboo (Figure 4.7) and with the percentage of screenings only at Port Pirie (Figure 4.8). Both of these sites were severely affected by a drier than average spring, suggesting that the Na excluding locus of Krichauff may only be of benefit under terminal drought conditions. Eight sites will be sown in 2004 to investigate further the level of tolerance conferred by this relatively small difference in Na exclusion, along with the soil and climatic conditions needed to record a significant yield difference.

The BC_3F_2 derived durum lines from the cross (Kalka#4*Na49) were grown at three sites in 2003, to assess the effect of this larger difference in Na exclusion (compared to that observed in the (Worrakatta*Krichauff) population) on field tolerance to transient salinity (Chapter 7).

The Redhill site failed to indicate any benefit of either boron tolerance or Na exclusion, despite having subsoil levels above those indicative of toxicity (Chapter 3, Table 3.1). Presumably the high pH of the subsoil was the dominant constraint. The yield of the Na excluding lines was only 93% of that of the non-excluding lines, presumably due to linkage drag. Fortunately, several Na-excluding lines out-yielded Kalka, indicating that deleterious genetic material was unlikely to be closely linked to the locus of interest.

Conversely, the Two Wells site provided positive evidence that Na exclusion has the potential to substantially improve the adaptation of durum wheat in southern Australia. At this site, the Na excluding lines yielded 27% more than non-excluders (Figure 7.7), while

the combination of boron tolerance and Na exclusion resulted in an increase in yield of 76% over the lines intolerant of boron and salt. This again emphasised the importance of combining all the traits for tolerance to the various subsoil constraints.

Breeding methods

The Na exclusion locus of Na49 has been shown to confer improved tolerance to transient salinity in the field. Hence, it is a priority for the trait to be introgressed into a wide range of varieties and breeding material, increasing the proportion of Na excluding lines available for use as parents. In view of the large benefit conferred by the Na exclusion locus of Na49 at Two Wells (Chapter 8) and the prevalence of transient saline subsoils in South Australia, this approach is expected to be as successful as breeding for boron tolerance.

An investigation into boron toxicity in South Australia (Paull, 1985, 1990) identified the bread wheat variety Halberd as having greater tolerance than other varieties. This variety was subsequently used as a donor parent in backcrossing programs to introgress the trait into a range of genotypes in the Waite wheat breeding program (Rathjen *et al.*, 1993). This program had previously been dominated by intolerant genotyopes, but after an intensive period of parent building though backcrossing, a large portion of the material segregated for tolerance to boron. Then the prevalence of boron toxicity at the sites where selection for grain yield was undertaken ensured that boron tolerant genotypes had a significant yield advantage, negating the need for screening (Rathjen pers. comm.).

While improvements in adaptation have resulted from subliminal selection by field testing large numbers of breeding lines, a far more efficient approach is to define the important

characteristics of the environment and follow a targeted breeding approach involving the following steps:

- define the constraint on production present in the environment,
- identify genetic variation for the tolerance to the constraint,
- incorporate the trait of interests into locally adapted material (parent building),
- intercross the upgraded parents with other advanced lines,
- select for agronomical suitability at locations at which the constraint is a major environmental factor.

The work described in this thesis and that underway at the Waite Campus to incorporate tolerance to the three known subsoil constraints (boron, transient salinity and high pH) into new durum varieties serves as an example for the improvement of other poorly adapted crops.

Other plant nutrients

The theory that reduced Na uptake will improve salt tolerance in members of the *Triticae* has resulted in the publication of many studies investigating the relationship between Na uptake and potassium (Greenway and Rogers, 1963; Gorham *et al.*, 1987; Gorham, 1988; Dvorak *et al.*, 1994; Dubcovski *et al.*, 1996; Munns *et al.*, 2000). It has generally been concluded in these studies that Na exclusion mechanisms operate by K/Na discrimination, or the selective uptake of K in place of Na. None of these studies have investigated concentration change of other elements in response to Na exclusion.

The BC_3F_2 derived population from the cross (Kalka#4*Na49) proved to be a useful tool for studying the effect of a very large reduction in Na accumulation on the accumulation of other elements (Chapter 8). While it was found that the accumulation of K^+ in the shoot

was significantly higher in Na excluding lines in all experiments, the concentration of other elements was also significantly affected. The uptake of the other cations Ca^{2+} and Mg^{2+} increased, while the anions Cl^- and SO_4^{2-} decreased. This suggested that the exclusion of Na^+ is balanced by an adjustment in the uptake of other ions, so the ionic and osmotic balance between plant and substrate is maintained.

In many cases the effect of Na exclusion on the uptake of other elements was the difference between deficiency and adequate nutrition at the field sites sampled. At several sites, the concentration of K, Mg and Ca in the non-excluding (commercial durum) genotypes was marginal to deficient, while the Na excluding lines had adequate nutrition. It may be that deficiency of these elements in durum grown on soils affected by transient salinity partly explains their poor adaptation to South Australian soils.

The effect of Na exclusion on the uptake of the micronutrients Zn, Mn and Cu is not yet clear, but the efficiency of uptake of these ions by durum wheat is known to be less than that of bread wheat (Cakmak *et al.*, 1996; Graham, 1988; Kaur *et al.*, 1989; Lewis *et al.*, 2001; Saberi *et al.*, 2001). It is also known that the availability of these elements is reduced on high pH soils, which commonly coincide with transient salinity.

Conclusion

Transient salinity has been shown to be a significant factor affecting cereal production in South Australia, along with high pH and boron toxicity. The Na exclusion locus from the landrace Na49 was found to improve the adaptation of durum to sites affected by transient salinity and is now being introgressed into a wide range of breeding material.

The subsoil constraints transient salinity, boron toxicity and high pH often occur in the same subsoils, so tolerance to one constraint will not necessarily ensure an increase in grain yield in the absence of tolerance to the other constraints. Consequently, combining tolerances to these three subsoil constraints is a high priority if the area of durum production is to be expanded.

The work currently underway at the Waite Campus to improve the tolerance of durum wheat to the subsoil constraints identified in southern Australia serves as an example of a suitable approach for improving the adaptation of all the locally grown crop species.

Appendix 1.

VARIATION IN LOCALLY ADAPTED WHEAT AND BARLEY VARIETIES FOR SALT TOLERANCE DURING GERMINATION

A1.1 Introduction

Variation for salt tolerance at the germination stage has been observed by many researchers (Epstein *et al.,* 1980; McQuire and Dvorak, 1981; Schaller *et al.,* 1981). As, germination tolerance is not related to salt tolerance during later stages of vegetative and reproductive growth (Ayres *et al.,* 1952; Richards *et al.,* 1987; Rawson *et al.,* 1988), the work undertaken in these Ph.D studies did not include research on the genetics and genetic variability for germination in saline soils.

While germination on saline soils is not a major factor limiting production in southern Australia, there are districts that occasionally exhibit patchy germination and crop emergence. The more severe and persistent of these patches of poor emergence are colloquially referred to by farmers as 'magnesia' patches.

The total area affected by a significant reduction in emergence is dependent on seasonal conditions. In years with adequate rainfall prior to seeding, salts are leached from the surface and diluted sufficiently to allow normal emergence on patches which had been bare in years with dry conditions at seeding. In the years following better emergence and growth, it becomes important to retain as much of the crop residue as possible to reduce surface evaporation. Mulch trials conducted by Kennewell (1999), showed that the application of cereal straw to existing bare patches could result in full remediation after one season, while untreated areas were unchanged.

Hughes and Jeffery (1995) estimated that 35,000 ha of South Australia were affected by magnesia patches, most of which is located on the upper Eyre Peninsula. This is comparable to the estimate of Kennewell (1999) of 45,000 ha (Figure A1.1). Similar areas were identified by mapping work undertaken by PIRSA Land Information (2001) under the leadership of David Maschmedt (Figure A1.2); however, this map does not identify the northern and eastern margins of the northern agricultural, which have been described as severely affected (Kennewell, 1999).

Figure A1.1. Distribution of agricultural regions of South Australia where areas affected by magnesia patches are located. Reproduced from Kennewell (1999).

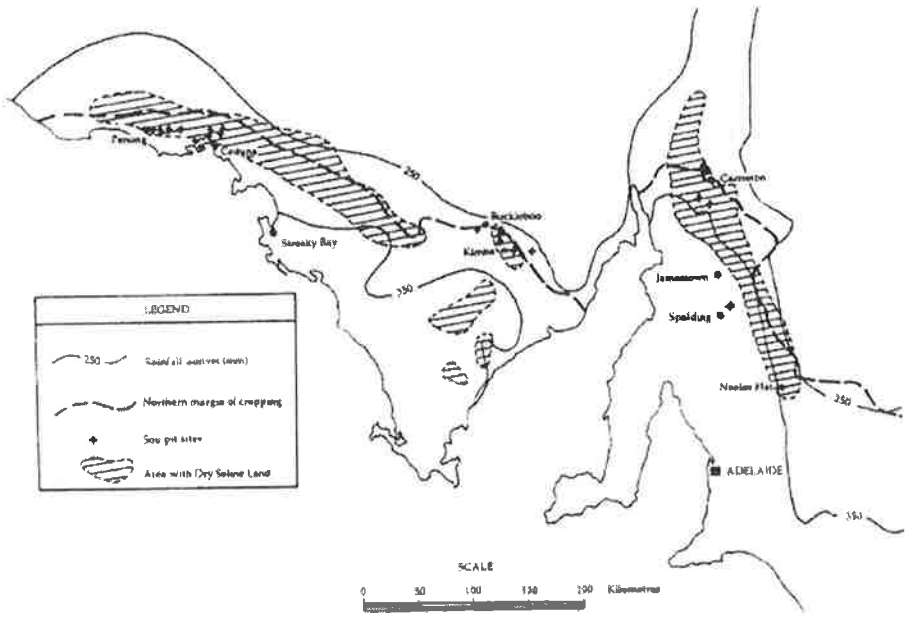
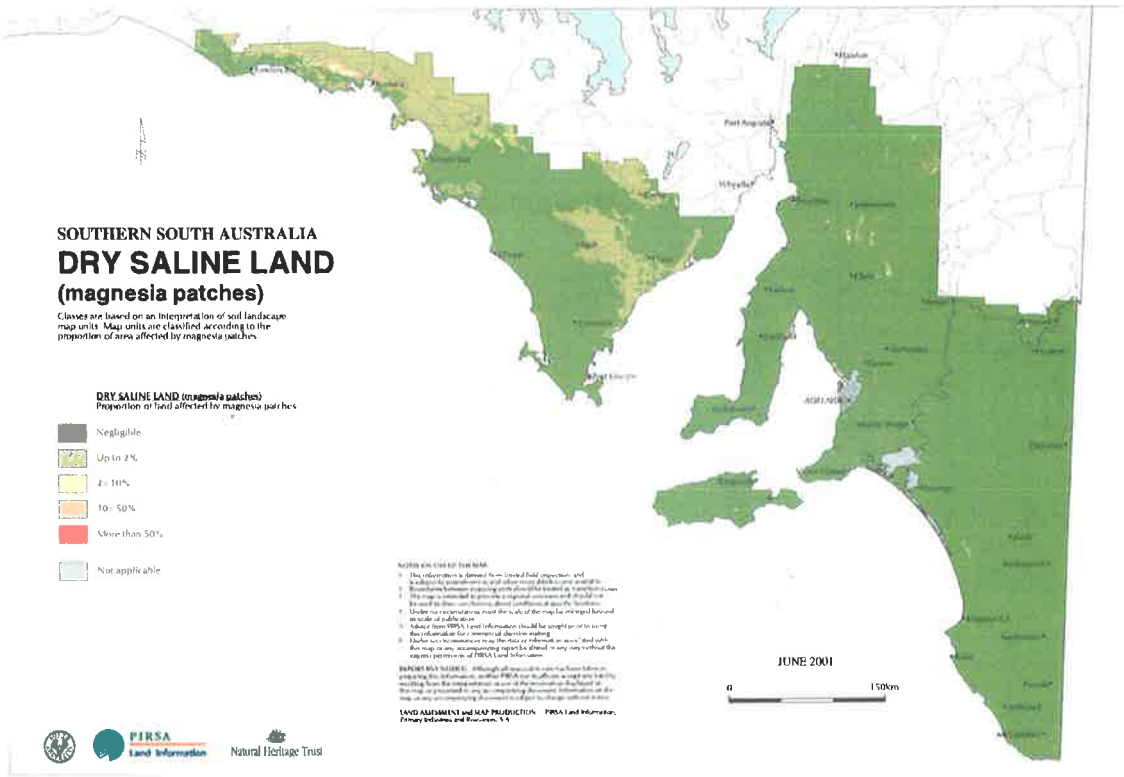


Figure A1.2. Distribution of areas affected by magnesia patches in the agricultural regions of South Australia. Reproduced from PIRSA Land Information (2001).



While the area affected by magnesia patches is not a large portion of South Australia's cropping area (possibly 1%), knowledge of varietal differences in tolerance would be useful for growers when choosing suitable varieties for sowing in affected paddocks.

An agronomy trial, including four varieties each of barley and hexaploid wheat, and two rates of DAP fertilizer (18:20:0, N:P:K) was sown on a site affected by transient salinity following below average opening rains. This resulted in very poor germination percentages and provided a unique opportunity to record genotypic differences in salt tolerance at germination.

A1.2 Materials and methods

The experiment was sown adjacent to the durum and bread wheat experiments described in Chapters 4 and 5 of this thesis, on the same day and using the same methods.

The experiment included four barley (Barque, Sloop, Keel and Chebec) and four wheat (Molineux, Krichauff, Frame and Westonia) varieties. These were combined factorially with two rates of DAP fertiliser (60 and 120kg/ha). All treatment combinations were replicated fourteen times into randomised complete blocks. The seeding rate of the individual genotypes was adjusted to aim at a plant density of 170 plants/m^2. The fertiliser treatments were sown with the seed, so that the fertiliser and seed were sown in the same furrow.

The emerged plant densities were obtained by counting all plants within a 1m length of plot (including all four rows). The number of plants was then multiplied by a factor of 1.667 to express the data as the number of plants/m^2.

An estimate of the level of transient salinity of each plot was obtained using the EM38 conductivity meter in the upright orientation, measuring mean soil conductivity to a depth of 1.5m. This data was used as a covariate in the analysis of variance of the plant emergence data.

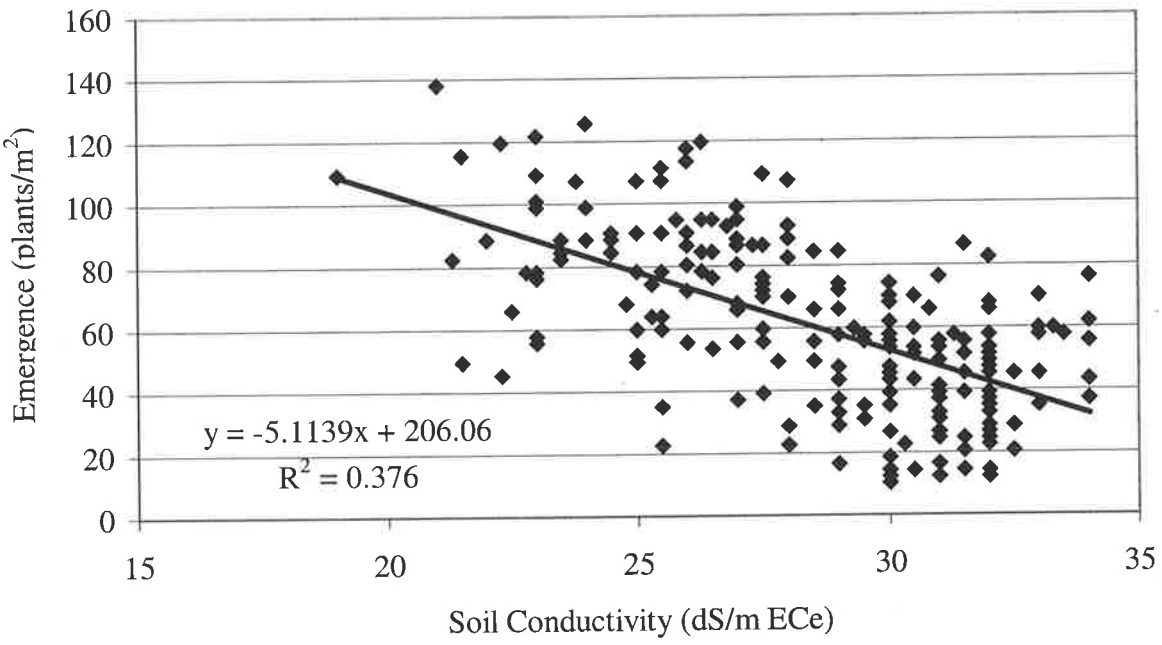
A1.3 Results

The mean conductivity of the soil was 28.6 ±3.2 dS/m ECe, while the mean emergence was 59.7 ±26.0 plants/m^2.

Analysis of variance of the emergence data revealed that the covariate conductivity had a significant (P<0.001) effect on emergence (Figure A1.3), which resulted in a reduction in plant emergence of approximately sixty percent between the low and high conductivity areas of the experiment.

Crop species had a significant effect on emergence (P<0.001), with wheat and barley having mean plant emergence counts of 52.7 ±12.6 and 65.7 ±12.6 plants/m^2 respectively. Fertiliser rate also had a significant effect (P<0.001), with the 60 and 120kg/ha rates resulted in mean plant emergence of 65.3 ±27.6 and 54.4 ±24.7 respectively. Despite the significant effects of species and fertiliser rate, the interaction between them was not significant.

Figure A1.4. The effect of conductivity (measured by EM38 conductivity meter) on plant emergence on a saline site at Port Pirie, 2003.



Analyses of variance of varietal effects were undertaken separately for the two crop species, to remove the possibility of significance being conferred by species alone. Analysis of the barley plots revealed that both variety (Table A1.1) and DAP rate had significant effects ($P<0.01$) on emergence, however the interaction between them was not significant. The covariate conductivity was also significant ($P<0.05$).

The effect of DAP rate was that the high rate resulted in significantly reduced plant emergence than the low rate, such that the mean emergence counts were 70.2 ±24.9 and 61.2 ±23.7 plants/m^2 for the 60 and 120kg/ha rates respectively.

Table A1.1. The plant emergence (plants/m^2) of four barley varieties on a saline field site at Port Pirie, 2003.

Variety	Plants/m^2
Barque	61.8 ±21.0
Chebec	57.1 ±24.5
Keel	71.7 ±30.3
Sloop	72.1 ±18.3

LSD (5%) 8.673

Analysis of variance of the plant emergence of the four wheat varieties showed that both variety and DAP rate were significant ($P<0.001$), along with the interaction between them ($P<0.001$). The covariate (soil conductivity) was not significant, presumably due to the magnitude of the effects of variety and fertiliser.

The variety x fertiliser interaction resulted from the varieties with the lowest plant emergence counts being more affected by the higher rate of DAP more than the varieties with increased emergence (Figure A1.5). This indicates that the effect of DAP was probably the same as that of salt and that both restricted emergence by decreasing the osmotic potential of the soil around the seed.

The variety Westonia had a higher rate of emergence than the other varieties on the saline site (Figure A1.5) and the higher DAP fertiliser rate had little additional effect. On the other hand, Molineux had the lowest number of emerged seedlings and also suffered the greatest reduction in emergence in response to additional DAP fertiliser (Plate A1.1).

Figure A1.5. The effect of wheat variety and DAP fertiliser rate on plant emergence on a saline site at Port Pirie, 2003.

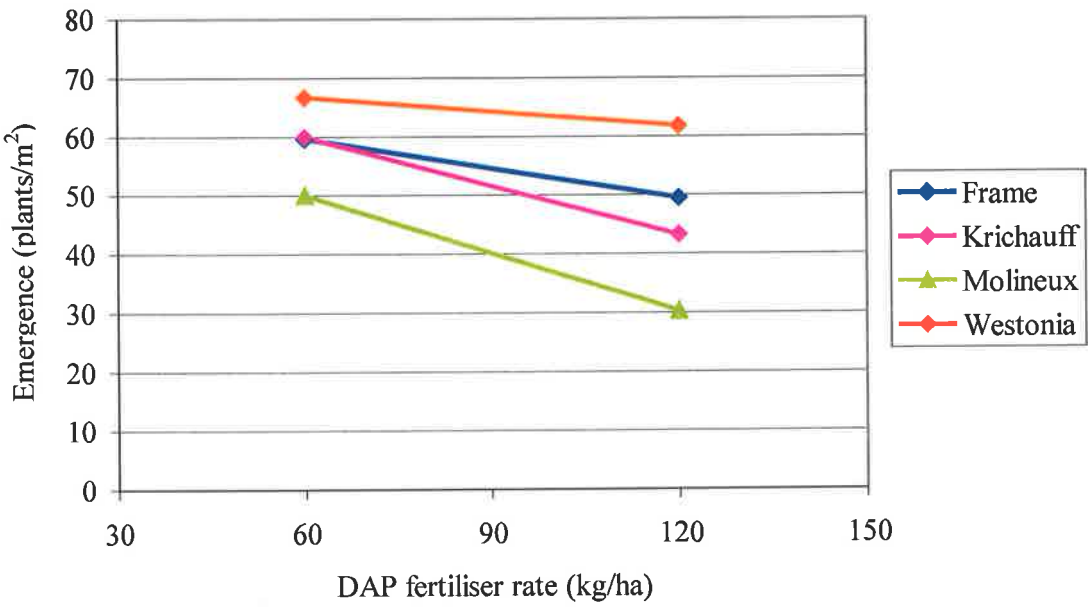


Plate A1.1. The effect of 60 and 120 kg/ha (left and right) of DAP fertiliser on the emergence of Molineux wheat on a saline site at Port Pirie (2003).

A1.4 Discussion

The results of this experiment indicate that the choice of crop species, variety and the rate of DAP fertiliser applied can have a significant effect on crop establishment on land affected by salt toxicity at the germination stage.

Historically, the rate of phosphorus fertiliser (DAP) applied to the saline patches would have been the same as that of the more productive areas of the paddock, while the removal in yield would have been far less. This would have resulted in a build up of plant available P in the saline patches of these soils, which could allow growers to reduce fertiliser rates on these areas, and consequently improve crop establishment.

Improved emergence and establishment will ultimately result in heavier crop residues, which act as a mulch, improving water infiltration and reducing evaporation from the surface. This will result in the salt moving down the profile, partially remediating the problem, although the same patches are likely to be affected by very high levels of transient salinity in the subsoil.

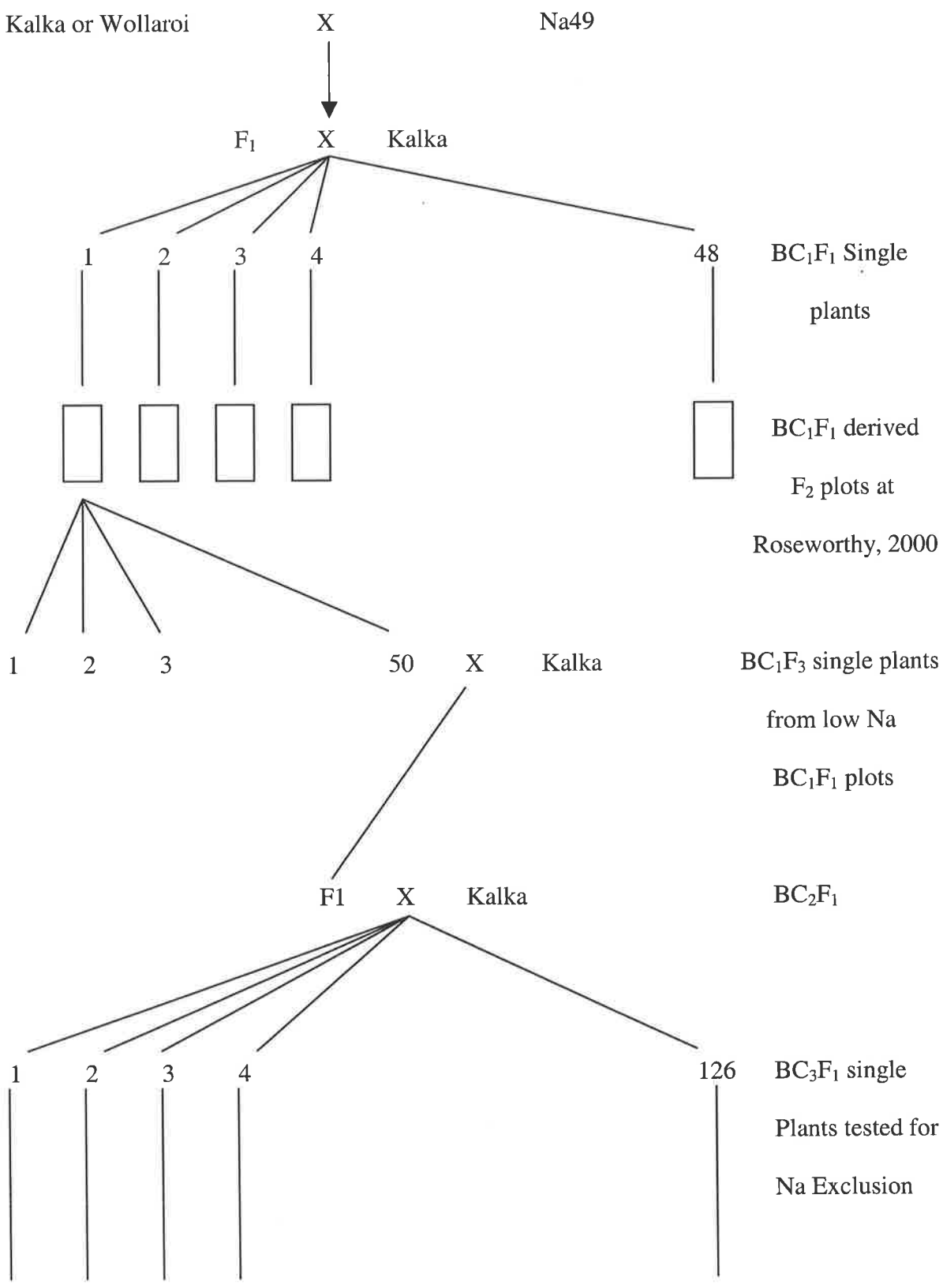
The choice of crop species is also important. If the affected patches are confined to specific areas of the paddock, it may be possible to 'patch out' the affected areas with barley. Alternatively, if a paddock to be sown to wheat has high surface salt concentrations throughout the paddock, it would be advisable to sow Westonia.

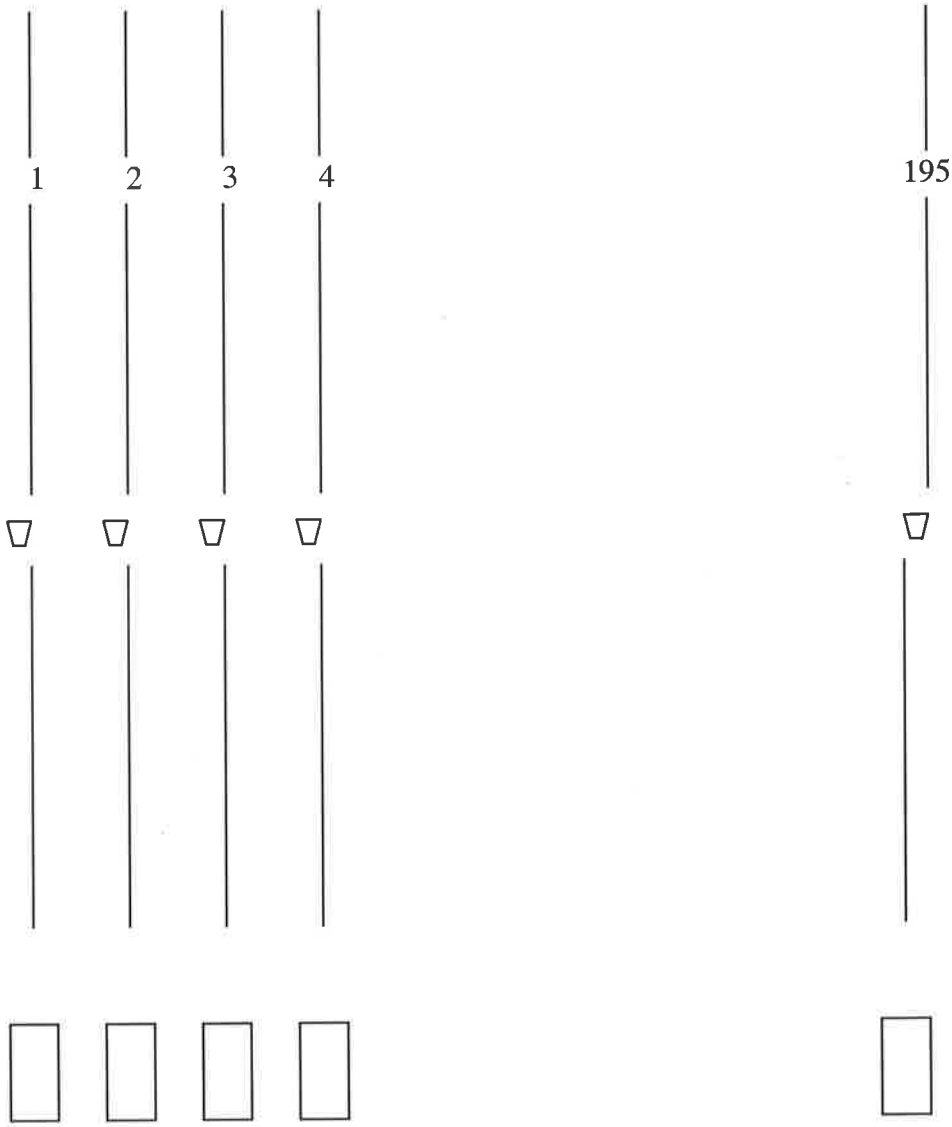
Many growers have recognised the ability of barley to emerge on patches where wheat cannot, and as a result sowing the worst areas to barley is a reasonably common practice.

Conversely, most growers were not aware that variation between wheat varieties existed and expressed substantial interest in the results.

Appendix 2.

PROCEDURE FOR THE DEVELOPMENT AND EVALUATION OF F_2BC_3 DERIVED LINES FROM THE CROSS (Kalka#*Na49).





BC_3F_2 derived F_4 field plots sown at Port Pirie, Redhill and Two Wells in 2003 with seed from BC_3F_2 derived F_3 multiplication rows grown in the bird proof enclosure at the Waite Institute. Plots were sampled for verification of glasshouse classification for Na exclusion.

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